17 December 2004

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Breakthrough of the Year

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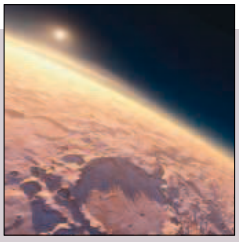

Breakthrough of the Year

Morning shadows darken Gusev crater, landing site of the Spirit rover, in this computer-assisted rendering of the ancient martian surface, based on topographic data from the Mars Orbiter Laser Altimeter onboard the Mars Global Surveyor. Discoveries by Spirit, its companion rover Opportunity, and the Surveyor spacecraft confirmed that some areas of Mars were once covered by shallow water and thus could have supported life. See the Breakthrough of the Year special section and the accompanying Editorial. [Image: Kees Veenenboss]

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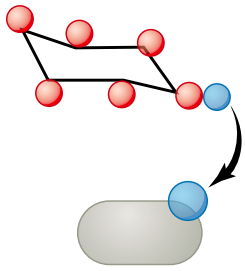

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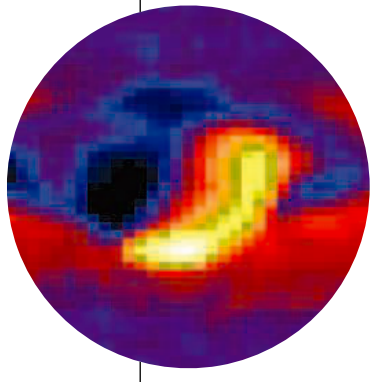

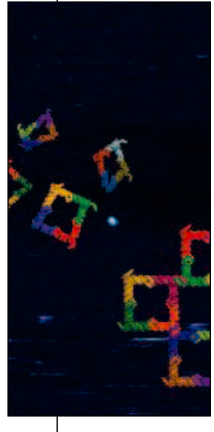

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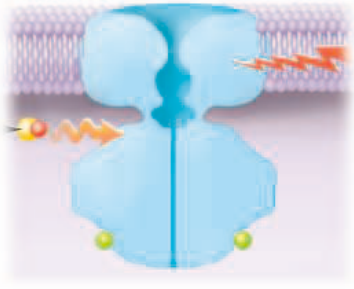

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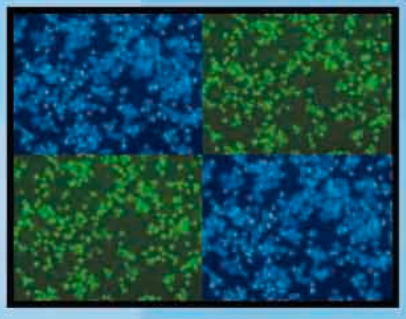

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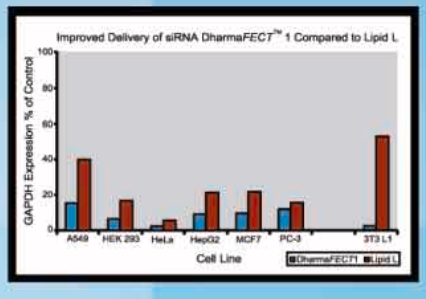

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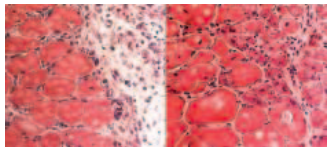

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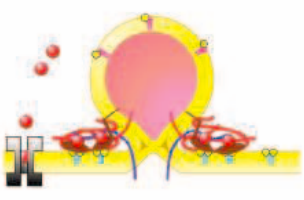

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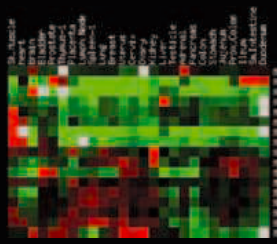

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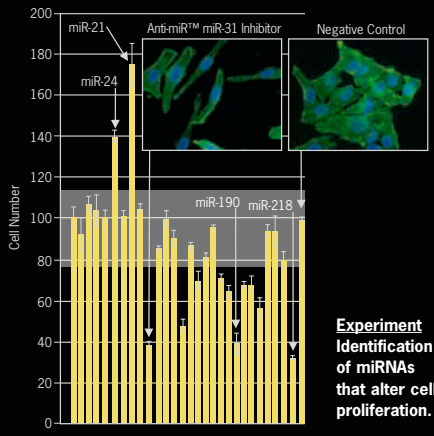

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edited by Stella Hurtley and Phil Szuromi

Cheating Heisenberg with Optical Combs

The Heisenberg Uncertainty Principle leads to tradeoffs when choosing between frequency domain and time domain techniques for spectroscopy. Frequency-resolved spectra measure energy levels with high precision, but the pulses are too long to probe dynamics directly. Ultrashort pulses can probe coherent behavior in state transitions but are too broad to measure state energies. **Marian *et al.*** (p. 2063, published online 18 November 2004) have exploited one of the properties of ultrashort pulses, which is that they are actually composed of many discrete frequency lines. The authors apply pulse-to-pulse phase stabilization, using the optical combs previously developed for frequency standardization, to spectroscopy. In a study of Rb atoms, they combine the frequency resolution of the narrow comb lines (for state energies) with the time resolution of the pulse envelope (for coherent dynamics). In addition, they measure and correct for the momentum imparted to the atoms by the light field.

DNA and RNA Swap Roles

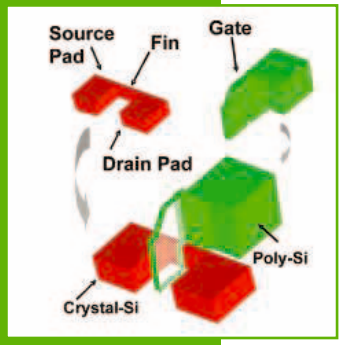
Two reports focus on the use of nucleic acids in creating complex material shapes and patterns and in directing molecular assembly (see the Perspective by **Yan**). Fragments of DNA can be designed that assemble into large-scale patterns and then be further functionalized or coated with metal particles. **Chworos *et al.*** (p. 2068) have now built a large library of shapes and patterns out of RNA, despite RNA's greater chemical lability. The authors start by constructing small- and large-sized tectoids, which are square in shape and that are designed with a variety of sticky tails at the corners. Three-dimensional periodic and aperiodic patterns can be formed from mixtures of the small and large shapes. The ribosome is an RNA and protein machine that strings amino acids into peptides specified by messenger RNA sequences. **Liao and Seeman** (p. 2072) have made a DNA machine that mimics some of the translational capabilities of the ribosome in that it can hook together sequences of DNA based on the way the machine has been set. The functional part of the device can assume two structural states, and is primed by short DNA segments that are not related to the sequence that the device assembles.

Ironing Out Sedimentary Origins

Some of the oldest rocks on Earth, dating to about 3.8 billion years ago, are found in southwestern Greenland, the Isua greenstone belt, and the related banded rocks on Akilia Island. Carbon isotopic data suggested that microorganisms helped to form some of these rocks in a sedimentary environment and thus represent some of the earliest evidence for life on Earth. Others argue that the rocks are not of sedimentary origin. **Dauphas *et al.*** (p. 2077) provide iron isotopic data which suggest that the banded quartz-pyroxene rocks on Akilia Island are of sedimentary origin and that it is likely that the iron was transported, oxidized, and precipitated from hydrothermal vents. The oxidation and subsequent isotopic fractionation could be produced by anoxygenic photoautotrophic bacteria, which would link these sediments with the earliest known life.

Toward Smaller Silicon Switches

One important measure of the size of transistors is that of the "gate"—the region in the device that actually blocks or allows the flow of current in response to changes in applied potential. Gate lengths are now at about 50 nanometers, but smaller devices cannot be made simply by scaling down the present architectures because of potential problems with leakage currents (an inability to turn the switch off) and capacitive losses. **Ieong *et al.*** (p. 2057) present an overview of strategies for creating transistors on chips with gate lengths below 10 nanometers, including the use of multiple gates and ways to speed up the flow of charge carriers in the gate region.

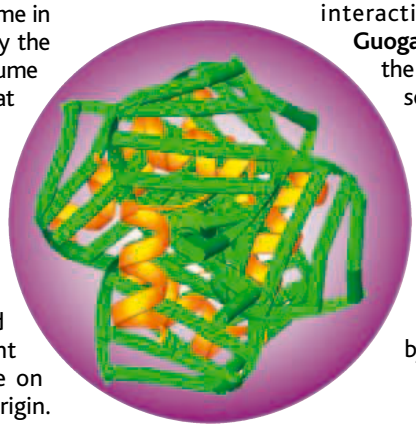


Love Thy Neighbor—or Thyself

In many plants, a particular gene system ensures that pollen from one plant is only capable of pollinating non-self plants, thus ensuring outcrossing. However *Arabidopsis thaliana* can self-pollinate. The genes that would normally enforce self-incompatibility, and thus outcrossing, still exist in *Arabidopsis*, but only as nonfunctional pseudogenes. **Shimizu *et al.*** (p. 2081) show that the sequence diversity found in these alleles through populations of *Arabidopsis* is considerably lower than found in active, self-incompatibility gene systems. In fact, the sequence diversity is so limited as to suggest the action of positive selection on these pseudogenes. Fixation of this transition to self-pollination has occurred recently, in evolutionary terms, perhaps when *Arabidopsis* ranges expanded after the Pleistocene. Self-fertility may prove useful to a species when it is expanding its habitat ranges.

The Beginnings of an RNA Virus Replication Complex

Many plant RNA viruses have a transfer RNA–like structure at the 3′ terminus of the viral RNA genome that is required for recruitment of the replicase. An exception is alfalfa mosaic virus, where the 3′ terminus comprises repeating hairpins separated by tetranucleotide repeats. The repeats bind to the viral coat protein (CP), and this interaction is required for replication. **Guogas *et al.*** (p. 2108) have determined the structure of a 39-nucleotide RNA segment bound to the N-terminal RNA binding domain of CP. Two CP peptides bind to sequential repeats in the RNA segment and the peptides and RNA co-fold into a defined structure. Such structural organization of the 3′ terminus may present a conformation that is recognized by replicase enzymes.



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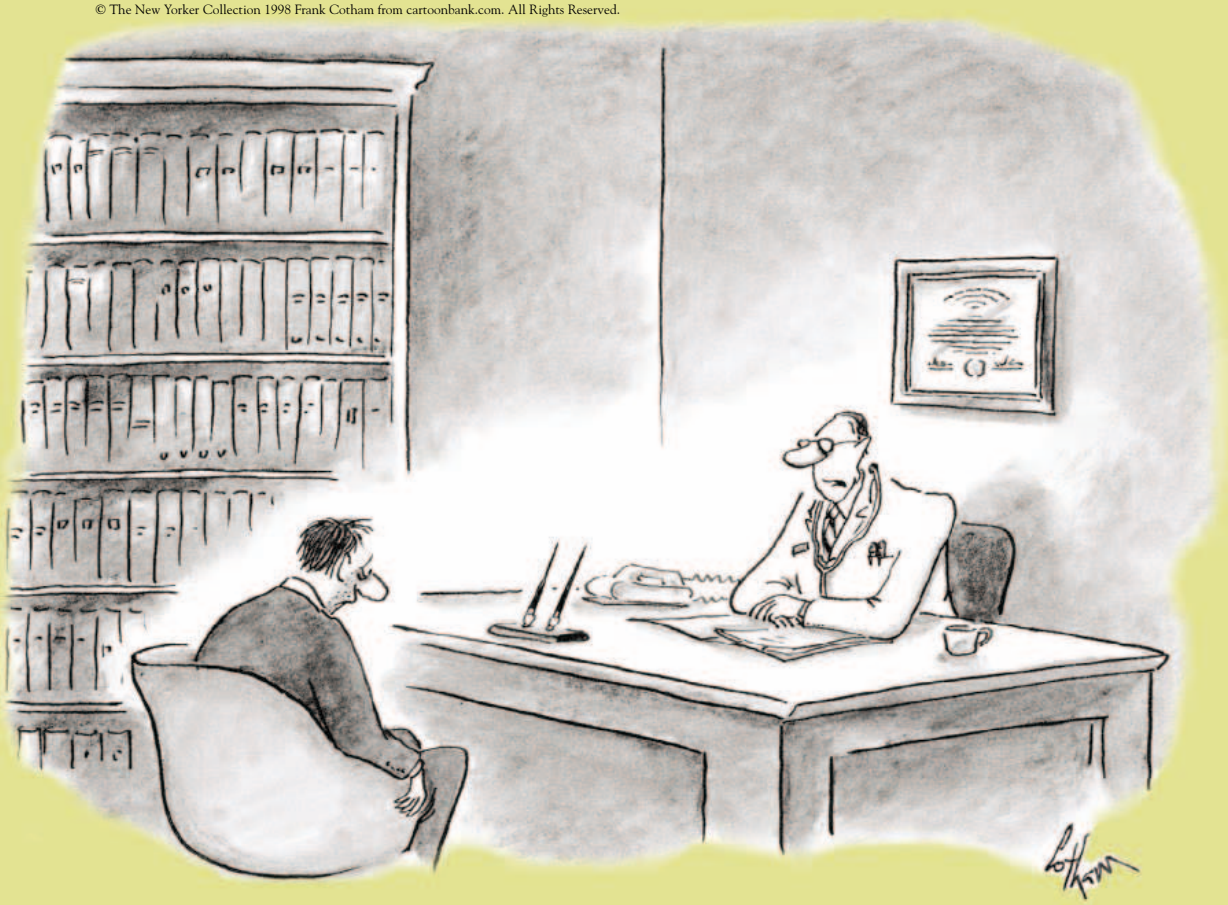

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Fundamentals of Iron Metabolism

The regulation of iron metabolism is a key component in maintaining health (see the Perspective by **Beutler**). **Nemeth *et al.*** (p. 2090, published online 28 October 2004) show that hepcidin, a peptide hormone produced by the liver in response to iron loading and inflammation, binds directly to the iron exporter ferroportin. Internalization of ferroportin leads to its degradation and prevents the export of iron from the cells. Iron overload diseases can be caused by the absence of hepcidin, and anemias can arise from increased production of hepcidin. Cells tightly regulate their responses to iron levels by using two proteins—iron regulatory protein (IRP) 1 and 2. Mice lacking IRP2 are severely compromised, but mice lacking IRP1 appear normal. **Meyron-Holtz *et al.*** (p. 2087) find that at physiological O_2 levels, cells lacking IRP2 misregulate iron metabolism, whereas in cells cultured in high levels of O_2—as commonly used in tissue culture—IRP1 can substitute for IRP2.

Every Breath You Take

The mammalian carotid body in the neck is a chemoreceptor that senses O_2 levels in the circulatory system and adjusts the respiratory rate accordingly. When O_2 becomes scarce, large-conductance calcium-sensitive potassium (BK) channels become inhibited, which causes cell depolarization and a cascade of responses that ultimately increases ventilation. **Williams *et al.*** (p. 2093, published online 4 November 2004; see the Perspective by **Hoshi and Lahiri**) now find that hemoxygenase-2 (HO-2) acts as an O_2 sensor to control BK channel activity. At normal O_2 concentrations, HO-2 uses O_2 as a substrate to generate carbon monoxide (CO), a critical channel activator. During hypoxia, when O_2 becomes scarce, HO-2 activity and CO generation fall, which inhibits BK channels and results in carotid body excitation.

Mitochondrial Maintenance Versus Induction

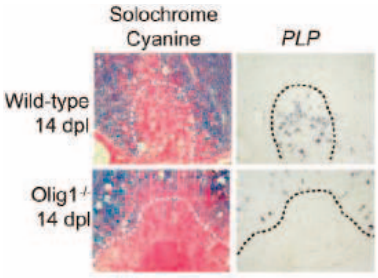
This replication of mammalian mitochondrial (mt) DNA is initiated at a number of start sites, or origins. **Fish *et al.*** (p. 2098) have identified an origin for mtDNA replication that is preferentially used by the cell under steady-state maintenance circumstances. The cell uses the other, previously described, origins after mtDNA has been depleted or when there are physiological demands for new mitochondria.

Back Door to Phosphorylation

Protein phosphorylation typically occurs through the catalytic activity of a kinase that transfers the phosphate moiety from adenosine triphosphate to a substrate. **Saiardi *et al.*** (p. 2101; see the Perspective by **York and Hunter**) show that the inositol pyrophosphate IP_7 can act as a phosphate donor to eukaryotic proteins. The nonenzymatic covalent protein modification was observed in cell extracts and in yeast cells. Because IP_7 and many of its targets have been implicated in various biological processes, this type of phosphorylation may represent an intracellular signaling mechanism.

Brain Repair Mechanism

The transcription factors Olig1 and Olig2 are closely related in sequence, but affect their key targets, oligodendrocyte cells, in different ways. Oligodendrocytes are responsible for wrapping neurons of the central nervous system in an insulating myelin sheath. Olig2 is important for developmental specification of oligodendrocyte cells. **Arnett *et al.*** (p. 2111) now show that Olig1 does not play a role in brain development but in repair. Mice lacking Olig1 are deficient in their ability to repair demyelinated brain lesions, the kind of lesions that occur in multiple sclerosis.



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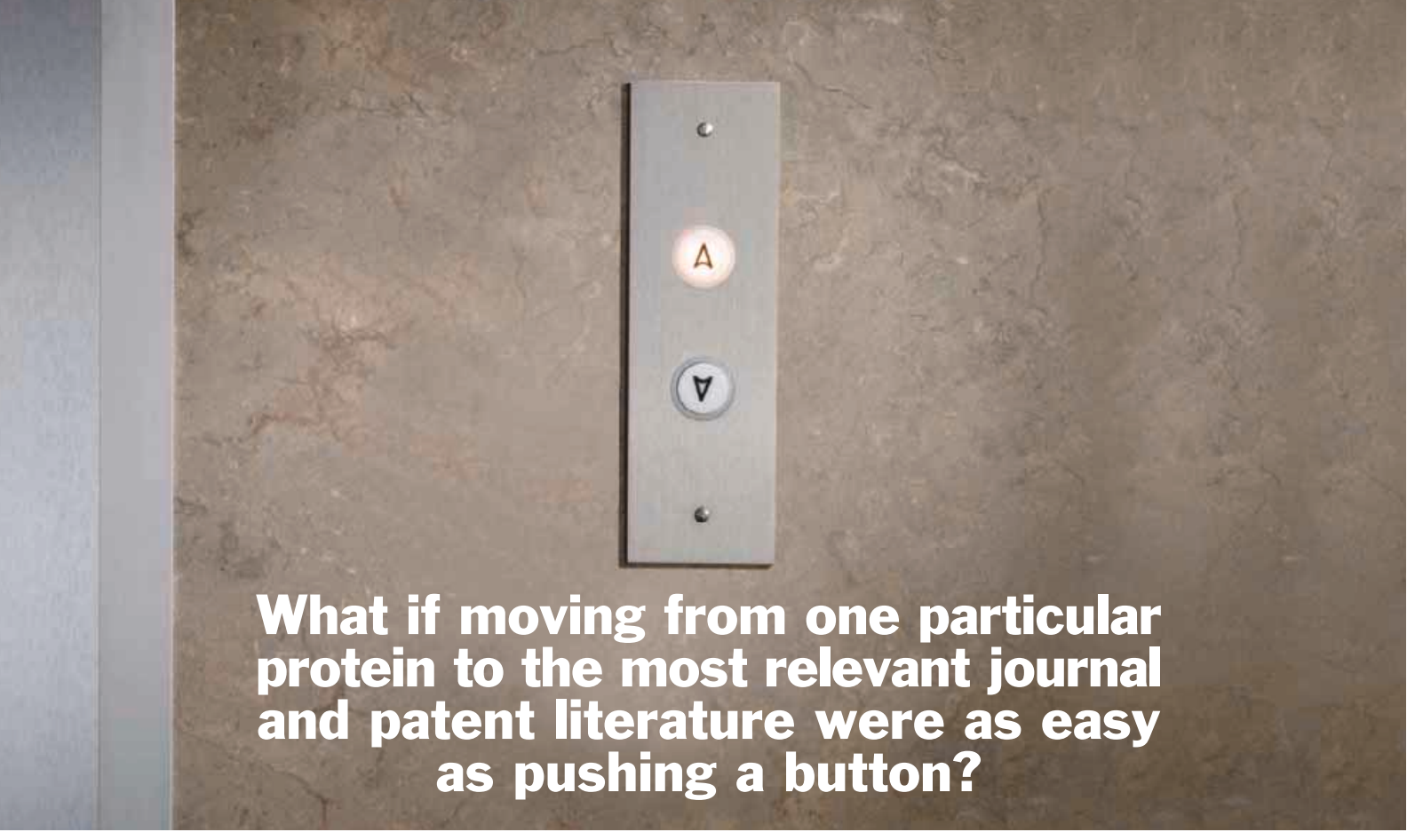

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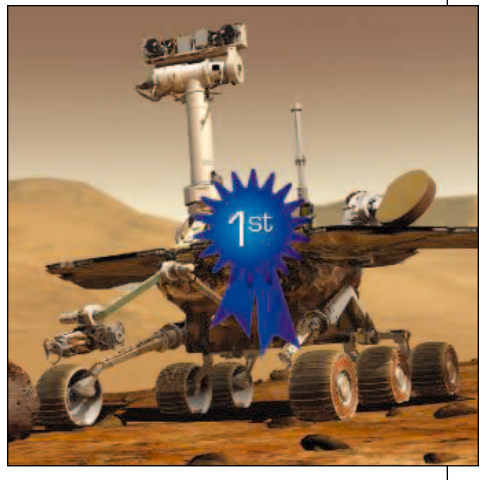
Breakthrough of the Year

Well, there wasn't much doubt about this year's winner. Unlike some past Breakthroughs, this one unfolded very much in the public eye, and the arguments that sometimes ensue when *Science*'s News and Editorial staffs converge for the selection were pretty tame this time around. The two Mars rover missions—well advertised by NASA beforehand—succeeded where two other recent attempts had failed, and succeeded spectacularly. The descent of the rovers held an enthralled audience of scientists and television viewers in suspense as they lit, took several pillowed bounces, and eventually came to rest.

The Breakthrough comprises the new evidence that Mars was once warm, wet, and salty: a candidate environment for early life. The emerging analysis, particularly from Opportunity, which landed amid Martian rock outcrops, confirmed that aquatic processes were responsible for depositing, forming, and altering rocks on a large scale on early Mars. The discovery is dramatic enough, showing what can be accomplished by a remote geologist with a good program. Of even wider significance is the demonstrated value of robotic technology—the real hero of the story—for a whole set of exploration and sampling tasks. Indeed, there is now serious talk of rescuing the Hubble Space Telescope with a robot. Other planetary sampling projects made the news in 2004 as well: Cassini, which will drop a probe to evaluate Titan's atmosphere in January; Mars Express, the European mission to sample the Martian atmosphere; Stardust, which sampled a comet; and Genesis which, despite crashing in Utah, seems to have returned with samples of the solar wind.



First place wasn't a headache, but picking the runner-up gave us a real challenge. The tiny hominin with the small brain, found on the island of Flores by an Indonesian-Australian scientific team, gripped the imagination of many. The finding that this was an island-dwarfed relict population of *Homo erectus* radically altered what we thought about human evolution. But it also raised questions: How could these primitive little people have coexisted for tens of millennia with big aggressive modern humans? (see the Perspective by Diamond, p. 2047). Controversy quickly arose, and the lone skull and related postcranial material are now under reexamination. We'll see how the story unfolds.

There were lots of other contenders. "Junk" DNA is being actively explored and yields a variety of riches: transposable elements, regulatory sequences, even codes for small RNAs. Other geneticists (some in companies, some in a well-funded public project) are mapping haplotypes: signature sequences in the human genome that may provide clues to ethnic history or disease liability. Astrophysicists were delighted by the discovery of a pair of pulsars orbiting in tandem: a system that may shed new light on these enigmatic spinning neutron stars.

Some of this we actually predicted in last year's Breakthrough issue in "Areas to Watch." We did reasonably well this time around. Mars activity led the list, and we called for a DNA data deluge (see above). We like our call on soil microbiology, and biodefense research did well, as predicted. But the controversy over open-access publishing resisted a clear resolution; and science and security, far from progressing significantly, remains a mess.

Each year, some disappointments ("Breakdowns") accompany the successes, reminding us that the scientific venture is fragile and dependent on public regard. Underscoring that point: This year's Breakdowns recognize a widespread crack in the social contract between the science community and the polity. That kind of disaffection was evident in Europe, as Italian scientists demonstrated to protest planned losses of tenure and French scientists went on strike to win some government concessions.

A Breakdown of a different kind was evident in the United States, where exchanges of tough rhetoric between the president's science adviser and a number of leading scientists made front-page news. Scientists objected, some of them on this page, to the use of political tests in the appointment of government science officials and the members of scientific advisory committees. There were sharp disagreements between many scientists and administration positions on stem cells and global climate change. And in more local and direct interactions with the American public, scientists faced a steady increase in challenges to the teaching of evolution in the public schools. It appears, alas, that this kind of tension is growing and that it may become a chronic feature of the landscape.

Donald Kennedy
Editor-in-Chief

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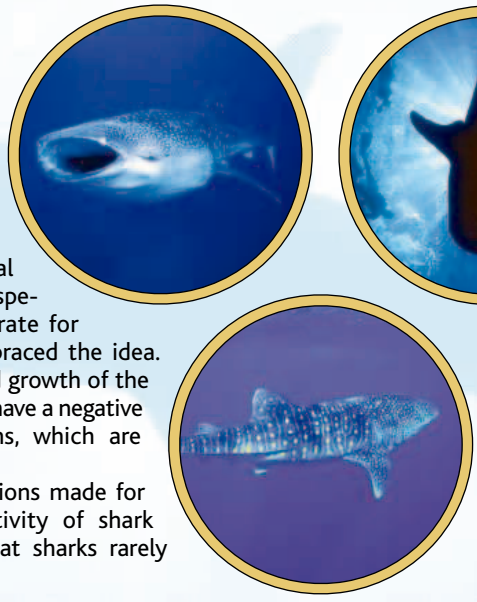
EDITORS' CHOICE

edited by Stella Hurtley

MEDICINE

Debunking a Fishy Tale



For more than a decade, shark cartilage has been touted as a rich source of anticancer agents. Although shark cartilage extracts have not yet shown efficacy against cancer in controlled clinical trials, the general public—especially cancer patients desperate for a cure—appear to have embraced the idea. Ecologists fear that continued growth of the shark cartilage industry could have a negative impact on shark populations, which are vulnerable to overfishing.

One of the main justifications made for studying the anticancer activity of shark cartilage is the assertion that sharks rarely develop cancer. Ostrander *et al.* describe evidence that this assumption may be incorrect. Gathering information from the National Cancer Institute's "Registry of Tumors in Lower Animals" and from the scientific literature, they identified 42 cases of tumors in sharks and their close relatives, about one-third of which were malignant. The authors point out the need for systematic surveys to determine the true incidence of cancer in sharks, and they discuss several alternative explanations for why sharks might have a low incidence of cancer, none of which require the presence of protective agents in cartilage.

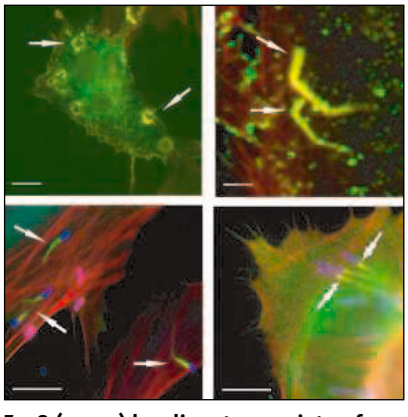
— PAK

Cancer Res. **64**, 8485 (2004).

CELL BIOLOGY

Capping the Barb

The propulsive force in cell motility is provided by the regulated growth of actin filaments. Actin filaments have a polarized structure with so-called pointed and barbed ends. It is the barbed end that is the site at which new actin subunits are added when actin filaments are forming in the cell, and this growth is regulated by proteins, exemplified by the protein gelsolin, that "cap" the barbed end. Disanza *et al.* now identify a new class of barbed end–capping proteins—in particular a protein termed Eps8, previously identified as a receptor tyrosine kinase substrate. Eps8 accumulates at sites where actin is showing dynamic growth. Reduction of the levels of Eps8 impairs actin-based motility. Eps8 contains an effector domain that caps actin and a domain that autoinhibits this activity. The autoinhibition is relieved by interaction with another regulatory protein: Abi1.



Eps8 (green) localizes to a variety of actin (red)– containing motile processes: phagocytic cups (top, left), and actin tails without (top, right) and with (bottom) associated bacteria (blue).

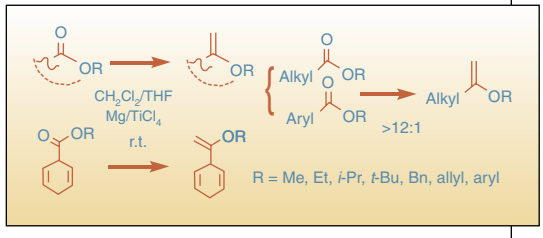
Croce *et al.* examined nematode worms that had been engineered to lack Eps8. Eps8 was found to be essential for embryonic development. Two isoforms of Eps8 were found, one of which, Eps8A, was specifically required for the apical morphogenesis of intestinal cells. The barbed end–capping ability provided by the C-terminal domain of the protein was important in promoting morphogenesis. — SMH

Nature Cell Biol. **6**, 1180; 1173 (2004).

CHEMISTRY

More Than a Solvent

Replacement of a carbonyl oxygen with a methylene (CH_2) group is often necessary in organic synthesis, but the typical methods for doing so involve sensitive reagents, such as highly basic ylides (Wittig reaction) or titanocene derivatives (Tebbe's and Grubbs' reagents). In a pair of papers, Yan *et al.* describe a convenient alternative system, based on a heterogenous mixture of TiCl4, Mg powder, and tetrahydrofuran, which uses the common solvent dichloromethane as the source of CH_2. The readily available reagents are simply mixed with aldehyde or ketone substrate, and the reaction proceeds within an hour.



Reaction scheme.

The nonbasic conditions tolerate a wide range of substrates, without disturbing acidic hydrogens or olefins prone to isomerization. Moreover, the reaction can proceed under severe steric constraints that block the titanocene systems. The second paper shows that increasing the Mg-to-TiCl4 ratio broadens the scope to include esters. — JSY

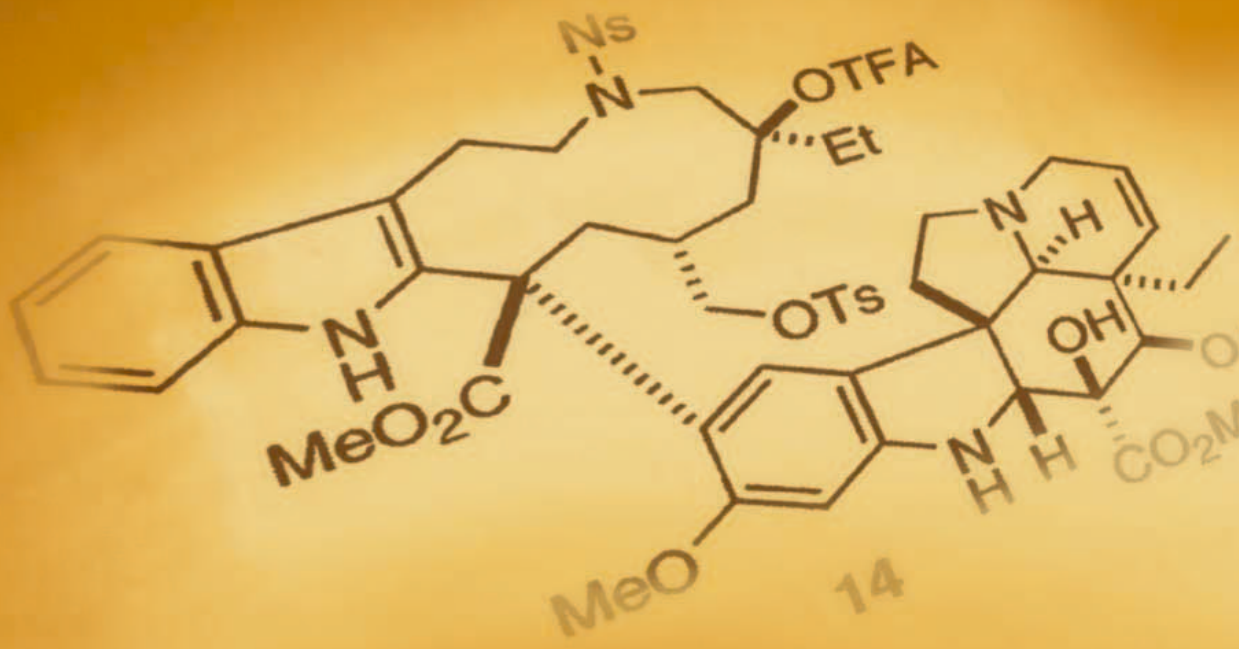
Org. Lett. 10.1021/ol0478887; 10.1021/ol047887e (2004).

BIOCHEMISTRY

Downhill from Here?

In the classical theory of protein folding, distinct native and denatured states are separated by an energy

CONTINUED ON PAGE 2005



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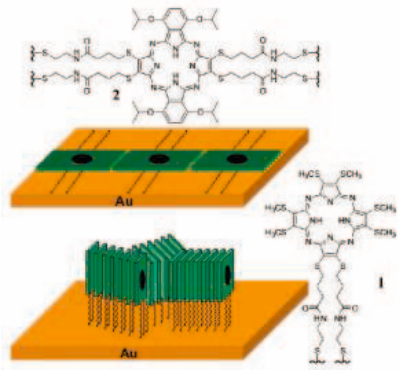
barrier, and transitions between the two are cooperative. An alternative model has been proposed in which the denatured state gradually merges into the native state as conditions change, with no significant energy barrier. Such downhill protein folding has been suggested for a fluorescently labeled version of the all-helical bacterial protein BBL (Garcia-Mira *et al.*, Reports, 13 Dec. 2002, p. 2191). Now Ferguson *et al.* suggest that the results may have been influenced by the labelling of BBL. Thermal denaturation of unlabeled wild-type BBL and two homologs was highly cooperative, with similar transition midpoints being obtained by a variety of techniques. In contrast, the introduction of extrinsic fluorophores into BBL complicated its unfolding behavior. Thus, downhill folding may occur for some proteins that do not have distinct folded states but is unlikely to be used by well-folded proteins such as BBL. — VV

J. Mol. Biol. **344**, 295 (2004).

CHEMISTRY

Reducing Is Easier When Lying Down?

The applied potential needed to oxidize or reduce molecules in solution reflects in part the energy needed to stabilize more highly charged species (ions versus neutrals). If the molecules are adsorbed on a metal, the formation of mirror-image charges should reduce the energetic expense of solvating a charged ion, because a dipole is formed instead. The effect of this on the coupling of the real and mirror charges should also drop off with distance. Vesper *et al.* provide experimental evidence for this effect using two porphyrazine derivatives adsorbed on gold surfaces. Derivative 1 has a single set of sulfur-terminated "legs" so that it



Schematic of the molecular orientations.

self-assembles in "standing-up" geometry, and derivative 2 was designed with two opposing sets so that would lie flat. The molecules were patterned on gold with dip-pen lithography, and the structures were verified by atomic force microscopy. In methylene chloride solution, the molecules showed similar redox behavior. When adsorbed on gold, the first ring-reduction potential of 1 shifted to less negative voltages by 0.43 V, whereas that of 2, whose central ring lies closer to the surface, shifted by 0.80 V. — PDS

J. Am. Chem. Soc. 10.1021/ja045270m (2004).

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Receptors on the Move

When T cells, B cells, and natural killer (NK) cells of the immune system interact with target cells, plasma membrane signaling molecules accumulate at the cell-cell interaction site: the immunological synapse. It seems that proteins, as well as signals, are transferred between the interacting cells at such contacts. NK cells receive inhibitory signals from cells that express self major histocompatibility complex (MHC) molecules on their surface, and the NK cells can actually acquire MHC class I proteins during these interactions with target cells. Now Vanherberghen *et al.* show that the exchange goes both ways and that NK receptors are transferred only to target cells that express MHC class I ligands. The NK cell receptor Ly49A was transferred only to target cells that expressed the cognate MHC class I ligand. It is not yet clear what function the transferred receptor might serve, but it is possible that the NK receptor might mark a target cell that has already been scanned by a NK cell. This, in turn, might allow more efficient surveillance by NK cells if they could recognize the marker and avoid rescanning the same cell. — LBR

Proc. Natl. Acad. Sci. U.S.A. **101**, 16873 (2004).

CREDITS: VESPER *ET AL., J. AM. CHEM. SOC.* 10.1021/JA045270M (2004)

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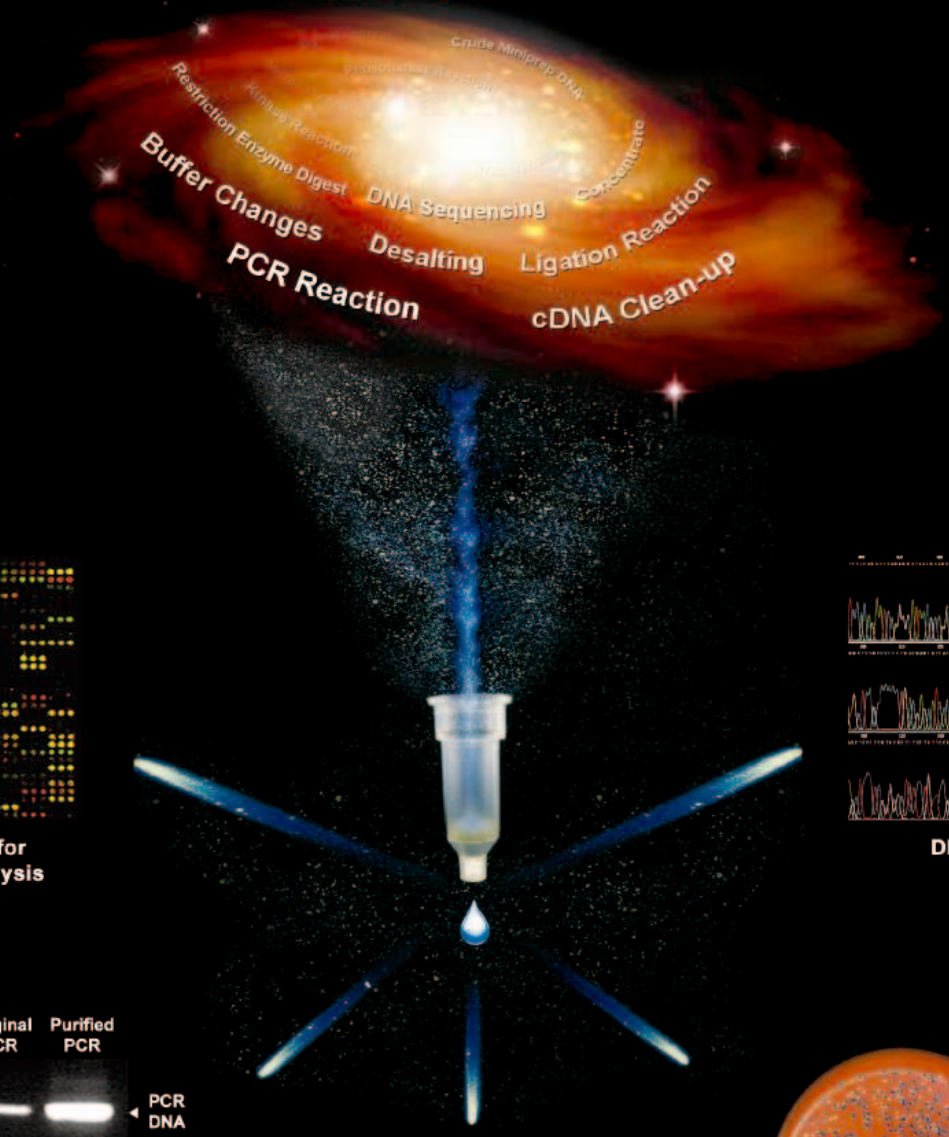

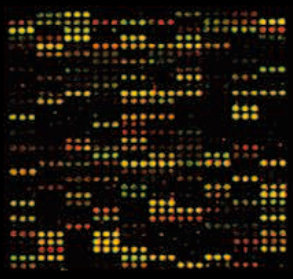

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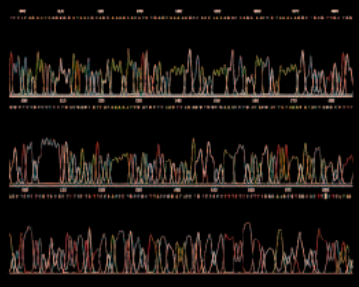

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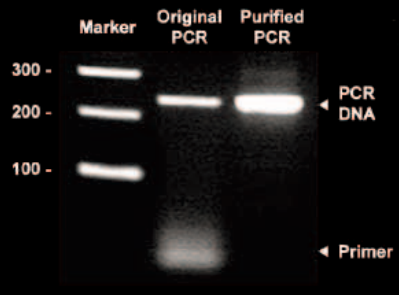

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NetWatch

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RESOURCES

Seeking Cnidarians

Who needs a brain? The cnidarians—corals, jellyfish, sea anemones, and their relatives—have stuck around for more than 500 million years without one. Researchers intrigued by these animals will find everything from stunning photos to genomic data at these two sites. Anthozoa.com,* run by German zoologist Vreni Häussermann, focuses on the group that includes corals and sea anemones. You can connect with fellow researchers by browsing a directory or joining a discussion forum. The site also includes a taxonomy of the group; species lists for Hawaii, the Mediterranean Sea, and other places; and several bibliographies. At left is the rare blue form of *Phymactis*, an anemone found from Peru to Chile.

Although the work lags behind genomic studies on nematodes and fruit flies, molecular biologists have been amassing data on sea anemones and their kin. At the Cnidarian Evolutionary Genomics Database, or CnidBase,† from Boston University, users can track down and compare summaries of gene expression studies gleaned from the literature for more than 20 species. The site, aimed at exploring cnidarian biodiversity, also lets you search for particular sequences in cnidarian DNA and find the latest genomics papers.

*www.anthozoa.com
†cnidbase.bu.edu

FUN

The Mathematician's Literary Companion

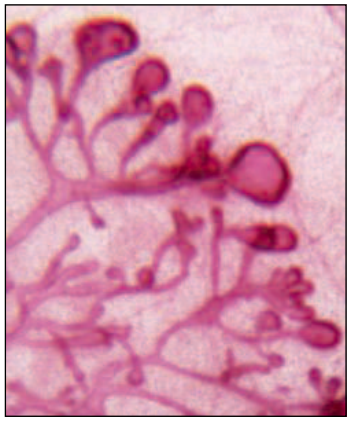
Every bookstore has a science-fiction section, but good luck finding the aisle devoted to "math fiction." Yet satirist Jonathan Swift, mystery writer Dorothy L. Sayers, macho filmmaker Sam Peckinpah, and many others have integrated math and mathematicians into their creations. Mathematical Fiction from Professor Alex Kasman of the College of Charleston in South Carolina lists more than 450 novels, short stories, comic books, and other works that feature math themes, characters, or examples.

As brief descriptions show, math can be tangential or fundamental to the pieces, and the portrayals of mathematicians range from sympathetic to scathing. For example, in *Gulliver's Travels*, Swift derides the hyperintellectual, math-obsessed residents of the flying island of Laputa: "in the common actions and behaviour of life, I have not seen a more clumsy, awkward, and unhandy people. ... Imagination, fancy, and invention, they are wholly strangers to." Visitors can comment on the choices or rate their literary quality and mathematical accuracy.

math.cofc.edu/faculty/kasman/MATHFICT/default.html

RESOURCES

Inside the Milk Gland



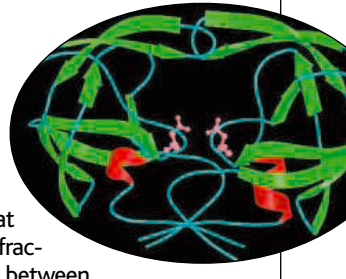
The eclectic Biology of the Mammary Gland site is aimed at developmental biologists, cancer researchers, and physiologists. The site, from Lothar Hennighausen's lab at the National Institute of Diabetes and Digestive and Kidney Diseases, includes everything from technical tips to pathology slides, mainly on mice.

A histology atlas brims with images of normal and unhealthy tissues. Visitors can track development of the mammary glands in mice from birth to pregnancy and compare the process in, say, mice lacking the estrogen receptor. Pages on techniques explain how to prepare and stain tissue, insert genes into mammary cells, and more. The reviews section includes slide shows, short backgrounders, and audio lectures on topics such as the physiology of milk secretion and breast cancer diagnosis. Above, branching milk-producing ducts from a 4-week-old mouse.

mammary.nih.gov

DATABASE

Protein Scissors



Up to 5% of proteins are peptidases, enzymes that split proteins by fracturing the bonds between amino acids. Peptidases perform many vital tasks, such as triggering blood clotting, but they also help viruses set uphouse inside their hosts and may promote illnesses such as Alzheimer's disease. MEROPS, hosted by the Sanger Institute in Hinxton, U.K., holds data on peptidases from more than 2300 viruses, bacteria, animals, and other organisms. The site organizes the entries into evolutionary lineages. Search for a peptidase such as HIV's retropepsin (above), which hews newly made viral proteins into usable lengths, and you'll get basic data on its classification and function. You can call up the enzyme's structure, the proteins it attacks, the organisms that make it, and a raft of references. MEROPS also boasts a database of mirror-image molecules that block peptidases.

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Breakthrough of the Year

THE WINNER

The Mars rovers, with the help of remote-sensing spacecraft, have sniffed out water and found the remains of one or more ancient environments where life could have survived. Indeed, early Mars is looking wetter and wetter

On Mars, a Second Chance for Life

Inanimate, wheeled, one-armed boxes roaming another planet have done something no human has ever managed: They have discovered another place in the universe where life could once have existed. Aided by other robots in orbit and a modicum of luck, the two Mars rovers earlier this year homed in on locales once rich with water. The Opportunity rover found the salty, rippled sediments of a huge shallow sea; the Spirit rover discovered rock once so drenched that it had rotted. Their finds mark a milestone in humankind's search for life elsewhere in the universe.

BREAKTHROUGH ONLINE

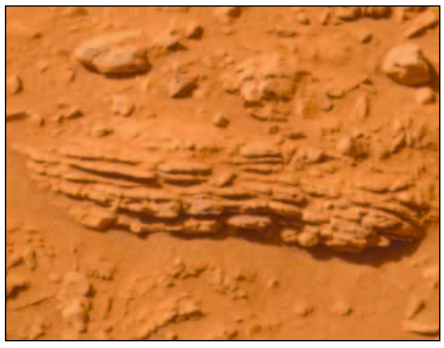
For an expanded version of this section, with references and links, see www.sciencemag.org/sciext/btoy2004

The two Mars rovers confirmed what many scientists have long suspected: Billions of years ago, enough water pooled on the surface of Earth's neighbor long enough to allow the possibility of life. Despite tantalizing hints starting with the Viking missions almost 30 years ago, Mars scientists could never be sure whether the water-carved valleys, channels, and gullies that they saw through orbiting cameras implied the prolonged presence of surface waters.

The Mars rovers have now put a bound on the water debate. Thanks to the hardy little robots, we know that Mars of several billion years ago was warm enough and wet enough to have a shallow, salty sea. This sea probably came and went, turning into wind-blown salt flats from time to time, but the puddles spanned an area the size of Oklahoma. Enough water passed through it to leave behind perhaps 300 meters of salt. And the dirty salt buried beneath its floor remained wet long enough to grow marble-size iron minerals.

On the opposite side of the planet, shallow groundwater also lingered long enough to transform hundreds of meters of what appears to have been volcanic ash into soft, iron-rich rock. And the latest spectroscopy from the newly arrived Mars Express orbiter shows that the salt from all this water-weathering of martian rock lingers in depressions elsewhere, sometimes in intriguing layered deposits that fill craters around the planet. For a time, it seems, early Mars was a watery, habitable place.

The Mars rovers didn't make their breakthroughs on their own. They had help from above. Opportunity needed guidance from the Thermal Emission Spectrometer (TES) on board the Mars Global Surveyor. TES



Rotted rock. The Spirit rover found this once-waterlogged rock that may have begun as volcanic ash.

was the first Mars-orbiting instrument to provide global coverage at infrared wavelengths where minerals leave distinctive signatures. The planet proved rather bland at TES wavelengths, but one area on the equator at the prime meridian was a glaring exception. The flat, dark Meridiani Planum jumped out as rich in gray hematite, an iron oxide. Researchers quickly came up with a half-dozen explanations for how gray hematite might have formed on Mars, most but not all of which involved water. None would prove entirely correct.

On arriving encased in protective balloons, Opportunity needed a couple of lucky breaks. First off, it stumbled—bounced and rolled, actually—into a geologist's perfect field site. As hoped, a small impact had exposed light-toned bedrock around the rim of its crater. This proved to be the long-sought evidence for prolonged surface waters. The booming hematite signal that drew the rover to Meridiani, on the other hand, actually came from marble-size "blueberries" of solid hematite that had weathered out of the sediment and now littered the ground as far as the rover could see. If the blueberries hadn't formed and been blasted out of the softer salt by windblown sand, TES never would have recognized the water signal.

Once on the scene, Opportunity could play field geologist to the hilt. Like Spirit, its identical twin on the opposite side of Mars, it came equipped with color-registering "eyes," a magnifying glass, a grinding wheel for exposing fresh rock, an elemental analyzer, and two mineral-identifying instruments: a remote-sensing "mini-TES" and a "hands-on" iron mineral identifier. With these tools, it set about unraveling the geologic story recorded in the curb-size outcrop of little Eagle crater.

Contrary to prelanding theorizing, Opportunity's story turned out to be about salt, an end product of the water weathering of rock, rather than the expected water-altered minerals. The Eagle outcrop is up to 40% salts, mostly magnesium and calcium sulfates. Much of the rest is "dirt," rock

CREDITS: NASA/JPL/CORNELL

Burns Cliff. The late Roger Burns predicted that volcanic acid would make Mars salty, like his namesake.

altered beyond recognition by water. The presence of the mineral jarosite suggests that the water was quite acidic, presumably from the sulfur dioxide of early martian volcanoes. Acid waters leached salts from martian rock and flowed across the floor of a shallow sea, or perhaps a vast puddle, permanently rippling the surface of the ancient sediment.

Then the water evaporated away, leaving the salt and dirt behind. The wind blowing across the salt flats sometimes blew the dirty salt into dunes. But beneath the surface, water persisted long enough to grow the hematite blueberries. The water came back time and time again, laying down centimeter-thick layers until 300 meters accumulated, judging by the light-toned outcrops in Mars Global Surveyor images. The salty sea or puddles appear to have spanned more than 300,000 square kilometers of Meridiani Planum. Only the orbiters' big-picture perspective could broaden Opportunity's findings this way, but only the rover could make sense of the orbiters' remote sensing.

Salty signs of long-past water were not confined to Meridiani. In Gusev crater, Spirit never did find any trace of the ancient lakebed inferred from orbital imaging. Instead, it roved across an ancient sheet of lava pulverized by impacts. But it did find volcanic rocks coated by weathering rinds and riddled with mineral-filled veins. Presumably, these rocks had once been buried in wet soil. By luck, Spirit overshot its intended landing site a bit, putting it within driving range of the 100-meter-high Columbia Hills. Orbital imaging had given no clue as to the origin of the hills, but Spirit found them to be one big pile of finely layered, water-altered rock.

Salt of Mars. Layered dirty salt (with hematite spherule) speaks of surface waters evaporating in ancient times.

While the rovers have provided the closest look yet at evidence of water on Mars, other instruments are rounding out the picture on a broader scale than two rovers can manage. Salty remains of water weathering have turned up in early surveys by the OMEGA spectrometer on the European Space Agency's Mars Express that went into orbit last 25 December. Largely because it has greater resolution than TES does, OMEGA found sulfates in ancient depressions such as the canyon network of Valles Marineris and in Meridiani. In Juventae Chasma, a branch of Valles Marineris, a 50-kilometer-wide, 2.5-kilometer-high, light-toned mesa sandwiches calcium sulfate between layers of magnesium sulfate.

So Mars was wet in its earliest years, when life on Earth was getting its start. But even then, Mars was taking a different environmental path, one too stressful for any life that might have managed to take hold. Even at Meridiani, the most habitable site found so far, the water was acidic, briny, and, at least at the surface, intermittent—not a promising place for life to originate. Still, life on Earth has evolved many forms that would survive and even thrive under such extreme conditions. The rover science team has called Meridiani Planum "an attractive candidate" for future landings. And given that both sulfates and iron oxides like

Doing Science Remotely

Take one and watch. "PillCam" includes a transmitter chip for beaming back views of the gut.

Most scientists start their careers with an urge to do hands-on science: to mix the chemicals, hammer off a chunk of rock, or feel the fevered brow. But scientists increasingly want to go where no one has gone before, or at least where no one can afford to go or would risk going: the surface of Mars, kilometers beneath storm-tossed seas, or the inside of your small intestine. Now, remotely operated or even autonomous machines are letting scientists keep their hands on things from inner to outer space.

The Mars rovers are the most visible in a long line of instrumented robots that have given planetary scientists a presence from innermost Mercury to beyond the edge of the solar system. No single component of a rover is a breakthrough technology like the ion propulsion that just delivered Europe's Smart-1 spacecraft to lunar orbit. Even when combined into a complex 174-kilogram package, the rovers' technology isn't very flashy. Their speed tops out at a nearly imperceptible one-tenth of a kilometer per hour, they can take a whole day to analyze one spot on one rock, and a pebble lodged in the wrong crevice can stop the show for days. But slow and steady wins the race in space. Although rover engineers predicted that both rovers would likely freeze to death in the depths of the martian winter last September, Spirit is still hobbling along with a couple of bad wheels, while Opportunity shows no serious signs of age.

On a far smaller scale, advanced technologies are making their way into inner space. Over the past 5 years, bioengineers have made considerable strides using telemetry, miniaturized sensors, and even self-adjusting instruments to keep track of the inner workings of the human body. The efforts enable doctors to follow their patients' progress more precisely, in real time, and sometimes from kilometers away. The innovations are affecting many medical disciplines.

For more than 20 years, doctors have been able to monitor pacemaker function remotely, but now these devices, which keep the heart beating regularly, can also detect whether their host is, say, running or sleeping and adjust the heart rate to its natural rhythm. Wireless pressure sensors implanted into repaired spines inform surgeons about the healing process, sensing the spine's increasing ability to bear weight. Other pressure-sensitive monitors fit inside the aorta to keep track of how well this artery is working. Still others fit into the eye to give feedback about pressure inside the eyeball, helping the physician know when to adjust medication. Bite-sized stand-alone cameras pass through the digestive system, sending images along the way. In particular, the camera captures what's going on in the small bowel, which otherwise requires invasive surgery.

More imaginatively, there's talk of "smart clothes": wearable electronics that track vital signs. Other devices may one day make sure patients take their medicines, sending a message via the Internet to warn physicians of noncompliance. Now that's hands-on.

–ELIZABETH PENNISI AND R.A.K.

CREDITS (TOP TO BOTTOM): NASA/JPL/CORNELL/U.S. GEOLOGICAL SURVEY; ZARLINK SEMICONDUCTOR INC.

hematite can preserve minute details of organisms, it could even be a good spot to find samples to send home to Earth.

Much remains to be done, however, before anyone picks a site for sample return. The leading geologic problem on Mars—the nature of light-toned, layered deposits such as those beneath Meridiani Planum and in Juventae Chasma—could be addressed by NASA's powerful Mars Reconnaissance Orbiter, to be launched in August 2005. The Phoenix lander will arrive in 2008 to study the role of present-day water ice on Mars. Because the planet's poles warm up dramatically every few tens of thousands of years, ice-rich soils there could host dormant life. And in 2009 NASA may launch Mars Science Laboratory, a hulking, far-traveling analytical lab on wheels that could pave the way for future sample return. With humans on Mars a distant prospect, the robots alone will be striving for the next Breakthrough of the Year on the Red Planet.

–RICHARD A. KERR

Scorecard 2003

Last year's forecasts of hot fields came close to the mark, on the whole.

Three on Mars. Two out of three isn't bad. The feisty Beagle 2 lander separated from the Mars Express mother ship handily but was never heard from again. But the two NASA rovers performed splendidly, and Mars Express itself is returning spectacular images. Opportunity easily found its prize, water-related mineralization, although the rock turned out to be a former salt flat rather than the expected hydrothermal deposits. As predicted, Spirit had trouble finding evidence of an ancient lakebed, which seems to have been covered by lava flows. In nearby hills, though, the hardy rover discovered something almost as good: volcanic ash once soaked and rotted by water.

Microbe militia. Biodefense flourished this year, as an estimated $7.5 billion flowed to efforts to develop everything from new drugs and vaccines to better sensors and new high-security laboratories. Gene libraries are filling up with data on potential bioweapons: Researchers completely sequenced the genomes of high-risk bacteria, such as anthrax, and have documented at least one strain of every virus and protozoan that might be weaponized. They've identified a cabinet full of promising treatments and started human trials on several new vaccines, including one for smallpox. But the government's new BioShield program, created to lure companies into the field, is off to a slow start, and critics say rules designed to keep bioweapons out of terrorists' hands continue to complicate research.

Genome data deluge. As predicted, the Internet is awash in genomic information. Chicken, rat, puffer fish, chimp, a red alga, and dozens of other genome sequences are now online, and dozens of researchers are comparing them in hopes of tracking evolution and pinpointing the causes of disease. Other researchers are busy building transcriptomes (broad looks at gene expression), interactomes (catalogs of protein interactions), regulomes (DNA elements that control gene function), epigenomes that explore nongenetic controls of gene function, and many other databases designed to illuminate how our genome works.

Open sesame. Efforts to make scientific information freely available over the Internet continue to grow—and so does controversy over who should pay the bill. In a major victory for open-access advocates, the National Institutes of Health is close to adopting rules that would require NIH grantees to make their papers freely available 6 months after they are published. Some publishers warn that the policy will sow confusion and financial chaos and may even bankrupt some journals. Meanwhile, open-access backers suffered a setback in the United Kingdom when the government declined to earmark funds to support free journals, concluding that it's still not clear the business model will prove viable.

Bottoms up. In 2003, physicists at the BELLE experiment in Japan announced a tantalizing hint of new physics in one particular decay of B particles. In 2004, however, new data have reduced the statistical significance of that result substantially. At the same time, lesser anomalies in other types of B decay keep the hope of new physics alive, so the issue has neither disappeared nor stood out in stark relief as predicted.

Digging deeper. More diverse and abundant than in any other ecosystem, the bacteria, viruses, and fungi under our feet have come to the fore in several fields: ecology, biodiversity, phylogeny, and environmental science. There's increased emphasis on the interactions between life below and above ground—for example, the effects of fungi on forest structure and the role of subterranean biodiversity on ecosystem health. These studies have driven home that the soil-microbe system is self-organized. The quest to understand this system has stimulated integrative studies that incorporate biochemical and biophysical, as well as biological, tools.

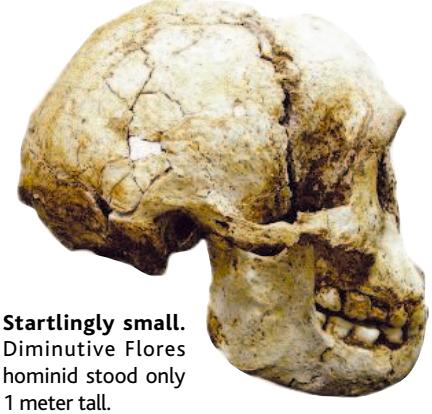
Science and security. Tightened U.S. security continues to give both American and foreign scientists fits, although some kinks in the new systems appear to be getting worked out. Surveys showed that enrollment of foreign graduate students at U.S. universities continues to slump, but fewer students are reporting visa-related delays. Foreign scientists are still having trouble making it to meetings in the United States, particularly on short notice, but many say border controls are improving. Several scientific societies, meanwhile, are suing the government over export-control rules that could make it illegal to edit papers submitted by researchers in a handful of "sensitive" nations. And some researchers are informally challenging agency decisions that put information once in the public domain—such as certain satellite photos and geological data—out of reach.

ILLUSTRATIONS: TERRY SMITH

THE RUNNERS-UP

2 THE LITTLEST HUMAN. Sometimes big discoveries come in small packages. In October, the startling news that a team of Indonesian and Australian researchers had found a new species of tiny hominid in a cave on the Indonesian island of Flores made headlines all over the world, and some researchers described it as the biggest discovery in half a century of anthropological research. If the team is right, the remains of *Homo floresiensis*, as the species was dubbed, suggest that modern humans shared Earth with other hominids as recently as 18,000 years ago. The skeleton's very small brain—a mere 380 cubic centimeters, compared with about 1400 cm^3 for *H. sapiens*—led its discoverers to hypothesize that it had evolved from an earlier population of *H. erectus* that got stuck on the island and then shrank in size to make maximum use of scarce resources.

Such "island dwarfism" is well known among other mammals—including small elephantlike creatures found in the same cave that the diminutive humans may have hunted with sophisticated stone tools. The discovery of *H. floresiensis* marks the first evidence that humans might also have been subject to drastic evolutionary pressure on islands. Many avenues of research suggest that throughout prehistory, humans followed the laws of evolution like any other creature, but this dramatic demonstration remains humbling for those of us who like to see ourselves as the masters of our own fates. Indeed, some skeptical researchers have found this claim of evolutionary downsizing too much to swallow and



Startlingly small. Diminutive Flores hominid stood only 1 meter tall.

suggest that the Flores hominid is really a pathological microcephalic modern human.

Just how quickly the debate is resolved remains to be seen, because the best way to solve it—analyzing still-unpublished fragments of other hominids found in the cave—is now threatened by a fresh controversy over who has the right to study the tiny remains. But the discoverers of *H. floresiensis* predict that there are many other small hominids on the islands of Indonesia just waiting to be found.

Pioneer. Woo Suk Hwang created a stir in February with the news that he and his colleagues had produced cloned human embryos.

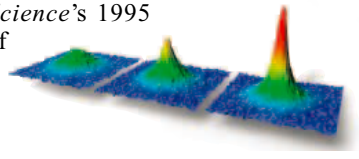
3 CLONE WARS. To tabloid readers, it might have sounded like old news, but the announcement by South Korean researchers that they had managed to produce a human embryo by nuclear transfer was the first scientific evidence that the technique could work with human cells. The researchers were not attempting to create a carbon-copy baby but rather to derive embryonic stem cell lines that could provide new insights into complex diseases or eventually produce replacement cells genetically matched to a patient.

Hundreds of mammals have been cloned since Dolly the sheep burst on the scene in 1997, but the psychological and political impact of the human work is still reverberating. It was the first evidence that cloning in primates is possible, contradicting earlier studies that had suggested that the location of cell-division proteins in primate eggs might thwart such attempts. Two factors were seminal: a gentler method of removing an egg's nucleus and a wealth of raw material. Sixteen young women donors provided 242 eggs for the project.

Eggs pose a key hurdle for those who hope to repeat the experiment. Several U.K.- and U.S.-based ethics boards have said scientists must rely on oocytes from failed in vitro fertilization attempts. Such eggs are scarcer and probably less robust than those freshly harvested from hormone-boosted ovaries.

The political impact of the work has been mixed. On 2 November, California voters, in part fueled by optimism sparked by the South Korean report, approved the creation of a $3 billion fund to support human nuclear transfer and embryonic stem cell work. But elsewhere, consensus has proved elusive. A United Nations debate over a worldwide ban on reproductive cloning ended in stalemate when countries that support the research could find no common ground with those that argue that all cloning research is immoral, in part because it creates embryos only to destroy them.

4 DÉJÀ CONDENSATES. It was another banner year for condensates, ultracold gases that display the signature of quantum mechanics writ large. The first condensates appeared in 1995, when researchers in the United States chilled a collection of atoms called bosons to the point at which they fell into a single quantum state, essentially behaving as one superatom. That achievement garnered *Science*'s 1995 Breakthrough of the Year. Over the past year, the condensate family tree has grown.



Howdy, partner. Signature of a fermi condensate.

Last December, physicists in the United States and Austria induced the other broad class of atoms, called fermions, to enter the realm of superatoms. To pull it off, the researchers had to induce fermions to behave like bosons. Bosons carry an internal angular momentum, or spin, with a whole-number value, a condition that allows them to share a single quantum state. But the spin of fermions is an integer plus one-half, which—thanks to the "exclusion principle" of quantum mechanics—prevents them from condensing, much as two negatively charged electrons repel one another when they get too close. The researchers wiggled their way around this inconvenience by inducing fermions to pair up into molecules with whole-number spin, which could condense just like bosons.

The discovery may shed light on one of the trickiest problems in physics: figuring out how electrons behave in complex materials, a key step toward a detailed description

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Areas to Watch in 2005

Recycling pays. It may be harder to pronounce than "apoptosis," but autophagy (self-eating) was on cell biologists' lips more and more this year. In autophagy, cells break down cytoplasmic molecules and portions of their membranes to provide nutrients during times of stress or starvation. After years in obscurity, the process has entered the limelight as scientists have identified genes driving it and used them to show that autophagy plays critical roles in cell growth and development, and even in disease. The momentum looks set to continue. A new journal, *Autophagy*, launches in January, and a Gordon Research Conference devoted to the area will be held in Italy in the spring.

Obesity drugs. As holiday meals once again lead people to vow to exercise more, biotech firms and pharmaceutical companies are racing to find a sweat-free alternative for our battle against obesity. More than 100 drugs targeting obesity are in the pipeline, and several should soon be submitted for Food and Drug Administration approval, especially since the agency has relaxed its guidelines to require only 1 year of safety data for such drugs. The most likely success story is rimonabant, which blocks the same brain receptors that marijuana tickles. Studies this year showed that it promotes long-term weight loss. As an added benefit, it may also curb the craving to smoke.

Big problem. Firms are racing to develop new drugs to help the growing number of obese people.

HapMapping along. The $100 million international Haplotype Map (HapMap) project is slated to wrap up toward the end of 2005—but it should bear fruit before then. The effort is developing maps built around haplotypes, shared stretches of DNA, in three populations: Utah residents with northern or western European ancestry; Chinese and Japanese; and Yoruban. Next year, the HapMap, along with a separate haplotype map assembled by the company Perlegen, may start to reveal the extent to which variation is involved in common human diseases and how DNA patterns shift across ethnicities. But the map's medical applications remain uncertain.

Cassini-Huygens at Saturn. The Huygens probe will likely make the biggest splash in planetary science in 2005, when it parachutes to the surface of Saturn's exotic, big moon Titan. Whether it will make an actual splash at the end of its 3-hour descent is anyone's guess. Cassini's haze-penetrating instruments have so far failed to find the postulated hydrocarbon seas, but Huygens should reveal the nature of the surface at one spot at least. The seven close Cassini flybys of Titan in the coming year could help clear up the mystery as well, but don't ignore the many upcoming Cassini passes by moons, rings, and Saturn itself.

Paper tigers. Are North Korea, Brazil, and Iran striving to develop nuclear arsenals? Conventional wisdom says yes, no, and maybe. Many analysts argue that North Korea's ultimate quest in six-way talks, expected to resume next year, is to bargain away its nuclear ambitions for economic aid and security guarantees. Brazil has barred inspectors from parts of its Resende facility, where it plans to enrich uranium for power reactors. Watchdogs are demanding more openness. After arduous negotiations with European officials, Iran last month agreed to suspend uranium enrichment while continuing to grow a nuclear power industry. In all three cases, the Treaty on the Non-proliferation of Nuclear Weapons has proven to be little more than a paper tiger; look for a revitalized campaign next year to strengthen the treaty.

European Research Council. This grassroots effort to create an agency to fund basic research across Europe gained political momentum in 2004. After endorsement by Europe's research ministers in November, it should take concrete shape in 2005. New European Union research commissioner Janez Potočnik has said he supports incorporating the idea into the Framework 7 funding program, which will begin in 2007.

Regulating nano. Nanotechnology is so broad that no single government agency is responsible for the field as a whole. So regulators in areas from consumer products, workers' health, and the environment are grappling with how best to ensure health and safety without stifling what is expected to be a major economic engine. Academic, legal, industrial, and government experts got a good start this year with meetings aimed at laying the groundwork for developing a standard nomenclature for the field and outlining the needs for research on nano's health and environmental risks. Progress should continue and broaden over the next year as countries strive to integrate their regulatory approaches.

of high-temperature superconductors. By tweaking their fermi condensates to vary the bonding strength between molecular partners, teams around the world systematically probed how their behavior changes as atoms grow farther apart. Already, such probing has revealed a key signature called a "pairing gap" similar to what is seen in high-temperature superconductors. Researchers also created the first supersolid, essentially a condensate in a solid. Because liquids had been condensed previously, researchers have now turned all three classes of matter—gases, liquids, and solids—into condensates.

5 HIDDEN DNA TREASURES. Biologists digging through the DNA between the genes and between a gene's protein-coding regions are unearthing new insights into how genomes work. Protein-coding sequences take up less than 10% of the human genome. The rest, previously considered a genetic wasteland, are proving quite influential for gene function. The wasteland is rich in genetic gems: short stretches of regulatory DNA, transposable elements (sequences that hop from one place to another), coding sequences that yield tiny RNA molecules, and so on.

By dissecting regulatory DNA, molecular biologists are learning about the exquisite controls that cause genes to turn on at the right time and in the right place. Short DNA sequences about 500 bases long, called activators, rev up gene expression by binding to regulatory proteins called transcription factors. Subtle differences in the arrangement of transcription factor binding sites cause gene activity to vary in different ways. Several reports this year have implicated activators as the source of genetic changes leading to the emergence of new species.

CREDIT: GETTY IMAGES

Junk DNA is chock-full of transposable elements. New work shows that these elements, when present between the coding regions of genes, can slow or halt transcription. They also help make new genes by hopping into existing ones, thereby altering the protein code. One such event involved a key gene for nerve function.

Junk DNA also encodes RNA, already shown to affect gene expression through RNAi (RNA interference). In yeast genes, for example, geneticists discovered that RNAi can block the binding of proteins needed to activate a gene involved in making the amino acid serine.

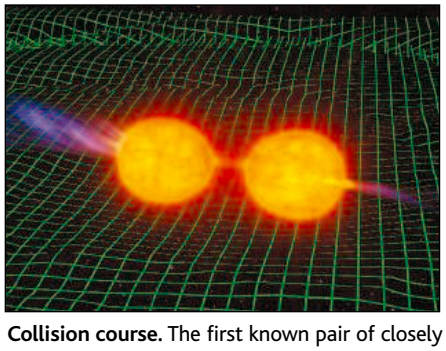
The quest to uncover more gems is revving up. The National Human Genome Research Institute has a new program, Encyclopedia of DNA Elements, that aims to capture and catalog all functional DNA within this "wasteland," starting first with 30 million bases of protein-coding and noncoding sequences.

6 PRIZED PULSAR PAIR. Astrophysicists doubled their pleasure this year by finding the first known binary system of pulsars: spinning neutron stars that whip tight beams of radiation into space. The system's properties have startled both observers and theorists, one of whom describes the discovery as a "watershed event" in the 36-year history of neutron star studies.

The pulsars turned up after the 64-meter Parkes radio telescope in Australia spotted an energetic pulsar, whirling 44 times every second, orbiting a hidden object that they presumed was a nonpulsing neutron star. Deeper scrutiny revealed that the companion also pulses at a leisurely rate of once every 2.8 seconds. But jaws dropped when the discovery team announced that the slower pulsar swoops almost directly in front of the faster one as they orbit in tandem, eclipsing the fast pulsar for nearly 30 seconds each orbit.

There's more. Blasts of particles and radiation from the fast pulsar distort the slow pulsar's magnetic field, making its radio signal flicker and nearly die out. Astrophysicists were thrilled because the eclipses and the complex interactions yield the first direct probe of the blazing plasmas in which pulsars turn on their mysterious beacons. Theorists sifting the clues say the intense wind of charged gas streaming from the fast pulsar may be nearly a million times denser than expected.

Researchers also expect the pulsar pair to provide the most stringent examination yet of Einstein's general theory of relativity. If any deviations from Einstein's theory exist, they are most likely to arise within the superstrong gravity of a neutron star or black hole. Astrophysicists are gauging the pulsars' motions as they gradually spiral inward toward an inevitable crash 85 million



Collision course. The first known pair of closely orbiting pulsars will merge in 85 million years.

CREDITS (TOP TO BOTTOM): TIM SMITH; LAURENT REBOURS/ASSOCIATED PRESS; JOHN ROWE ANIMATION

Breakdown of the Year: The Unwritten Contract

For more than a half-century, U.S. academic scientists have thrived on a tacit promise from the federal government to support their research in return for working toward the public good and training the next generation of scientists and engineers. Relationships between the government and scientists have occasionally been strained, especially when budgets have been tight, but in general the system has operated in a relatively civil manner. And it has worked well enough for other countries to try to copy, with mixed success.

But in 2004 that social compact took a beating. Groups of researchers accused the Bush Administration of undermining the scientific advisory system and of putting ideology before science in a number of issues from global warming to stem cell research. That elicited a strong rebuttal from the president's science adviser John Marburger, who dismissed a letter from 60 Nobel laureates criticizing the Administration's science policies as "complaints from the Democrats."

Sign of the times. The unhappy message displayed on a poster of Nobelist Marie Curie—"They are getting crazy, let's rescue research"—during a Paris street protest earlier this year reflects a growing tension between researchers around the world and their governments' science policies.

The United States wasn't alone in witnessing this breakdown of comity. In France and Italy, researchers staged a yearlong series of protests against what they viewed as attempts to undermine the scientific enterprise, from budget cuts to the proposed elimination of tenure. Across Europe and Asia, scientists felt the sting of activists denouncing work on genetically modified crops or research involving animals. And back in the United States, educators continued to battle antievolutionists seeking to influence science instruction in public schools across the country.

The scientific community bears some of the blame for this breakdown. The letter writers' overt sympathies for the Democratic nominee, Senator John Kerry, made them vulnerable to countercharges that they were also putting politics and ideology before science. The well-documented sclerosis within the French and Italian research establishment is largely self-induced and can't be cured with slogans and street demonstrations. And when a scientific issue rose to the level of a national debate, as in the controversy over the use of embryonic stem cells in research, the tendency of scientists to view their critics as biomedical Luddites left little room for compromise.

Ironically, politicians have long urged scientists to become more active in the policy arena. But this year was a reminder that there are risks involved, too. As Congress and the Administration look for ways to trim spending next year, scientists will need more friends in high places. And that means finding ways to make peace, not war, with the powers that be.

—JEFFREY MERVIS

Avian Influenza: Catastrophe Waiting in the Wings?

It's still primarily a bird disease, known to have killed only 32 humans since January. But H5N1, the avian influenza strain that swept across eastern Asia in 2004, killing millions of poultry, has cast a darker cloud over human health than numbers alone can explain. Experts fear that the virus could spawn a new influenza pandemic—a public health disaster of potentially devastating proportions. As Asian farmers saw their livelihoods destroyed this year, scientists made one worrisome discovery after another about the virus, and public health authorities around the globe began to take the risk seriously—only to discover that, should a pandemic erupt tomorrow, the world would be pathetically ill prepared.

Early this year, some believed that the outbreak, which started late 2003, might still be contained by mass culling of infected and exposed birds. This strategy worked well during the first known H5N1 outbreak, in Hong Kong in 1997, and the 2003 explosion in the Netherlands of H7N7, another bird flu strain. That hope is now gone; the virus is too entrenched and the affected area too large for eradication to be feasible. Researchers also discovered that ducks, which often mingle with chickens on small Asian farms, can harbor and shed large amounts of the virus without getting sick, perhaps creating an important, almost intractable reservoir.

The realization that H5N1 is here to stay has led to several shifts in strategy. One is the growing acceptance of the idea of protecting flocks through vaccination. Traditionally, animal health experts have preferred to stamp out bird flu, as they do for many viral diseases, because vaccination can enable the virus to continue circulating below the radar screen and ignite new outbreaks; it can also lead to costly export restrictions. But vaccination has now been added to the armory of weapons to fight H5N1 in several countries.

Bye bye bird. Despite massive poultry culling, the H5N1 flu strain seems here to stay.

With respect to human health, H5N1's long-term presence has put the risk of a new pandemic—a phenomenon unseen for 36 years—on the scientific and political agenda. Pandemics arise when new flu strains, to which nobody is immune, evolve ways to replicate easily among humans. In theory, this can happen with any number of strains, but the sheer scale of transmission has now made H5N1 a prime candidate. Adding to the concerns is H5N1's unusually broad host range (it has been shown to infect mice, cats, and tigers, for instance), its high mortality rate among known human victims, and one apparent case of human-to-human transmission in Thailand.

Nobody knows how likely a pandemic is or what its consequences would be. Past experience offers little to go on; pandemics in 1957 and 1968 were relatively mild, whereas experts put the death toll for the "Spanish flu" of 1918–19 at anywhere between 20 million and 100 million. (The world's population was less than 1.9 billion at the time.)

The World Health Organization is urging countries to draw up plans for how to cope, and some—mostly in the developed world—have begun to do so. But the challenges are enormous. A new vaccine would take many months to develop and mass-produce, and most countries don't have that capacity. (Even production of the annual flu vaccine is fragile; a glitch at a British plant almost halved the U.S. supply this year, creating instant shortages and chaotic situations.) Antiviral drugs could help bridge the first months. But few countries are stockpiling them, and many could never afford that option.

Experts say 2004 may well prove to be a pivotal year: one in which the danger multiplied and the world woke up. Time will tell whether it slumbered for too long.

–MARTIN ENSERINK

years from now. These measures—aided by the ultraprecise clocks of the pulsars themselves—may reveal the density and distribution of matter within a neutron star for the first time.

7 DOCUMENTING DIVERSITY DECLINES. From frogs to butterflies, ecologists and environmentalists outdid themselves this year in quantifying peaks and valleys in biodiversity. Disturbing news has come from large studies that show real declines in species richness.

Five hundred herpetologists completed the first global assessment of amphibians, and the news was not good. At workshops hosted by Conservation International and the World Conservation Union, researchers presented data on all 5700 known amphibian species. They concluded that more than 30% were vulnerable to extinction, and some were critically endangered. Half these species might disappear over the next century, victims of overharvesting, loss of habitat, and unknown causes.

Going, going ... This leopard frog is losing ground.

Naturalists who have tracked butterflies, plants, and birds in the United Kingdom for up to 40 years also turned up sobering statistics. Annual surveys in 10-kilometer quadrants showed that on average butterflies had disappeared from 13% of the squares. Researchers calculated that 71% of butterfly species had lost ground. Systematic counts of bird species in the U.K. showed that their numbers had dropped by half.

That work also found that 28% of the native plant species had disappeared from at least one square. Another U.K. study took a systematic look at grasslands growing on nutrient-poor soils. It revealed that species richness drops as the deposition of inorganic nitrogen—a product of industrial processes—increases. In some cases, the number of species declined by 23%.

Diversity data far beyond the British Isles came from a compilation of 40 ecological studies. Lasting 2 to 5 decades, these efforts turned up 20 places where warming had changed the natural history of those areas. For example, red foxes are showing up north of their territory, barging in on Arctic foxes. Plants are flowering earlier. Birds are changing their migration habits and settling in places where food supplies have already peaked.

Bottom line: Biodiversity continues to be in trouble.

CREDITS (TOP TO BOTTOM): USGS; DOAN BAO CHAU/ASSOCIATED PRESS

8 SPLISH, SPLASH. After a century of intense scientific study, water still gives researchers much to scratch their heads about. This year, a flurry of papers on the structure and chemical behavior of this familiar substance revealed results that, if they hold up, could reshape fields from chemistry to atmospheric sciences.

First and most controversial, a team of researchers from the United States, Germany, Sweden, and the Netherlands reported that the 100-year-old picture of the structure of liquid water might be wrong. Theorists thought slight charge differences between oxygen and hydrogen atoms pulled liquid water into an extended network, with each water molecule bound to four others in a tetrahedral pattern. But the team's synchrotron x-ray results suggest that many water molecules are, in fact, bound to only two neighbors. Don't rewrite the chemistry textbooks just yet: More-recent x-ray data back up the original structure, and debate will likely rage through 2005.

Another dispute centers on where ions in a large body of water hang out. Do they reside at the surface or get sucked into the interior? Conventional wisdom says electrostatic forces at the water's surface repel ions that are abundant in seawater, forcing them to go deep. But researchers tracking sea salt particles in the air over Los Angeles say the particles are so rich in halides (chemical relatives of fluorine) that those ions must be present on the water's surface. This year, computer simulations supported the idea. If true, atmospheric scientists may have to ponder new types of chemical reactions occurring on the surface of aerosol particles.

New experimental techniques are solving other mysteries. In April, a team in California reported that firing femtosecond bursts of electrons at water on a silicon surface had revealed crystallite-like ice structures that help bind water to the surface. And other groups used improved methods for making and tracking water clusters to determine how electrons and protons dissolve in water, providing new insights into aqueous chemistry. At this rate, water researchers won't be swimming in circles 100 years hence.

All wet? Synchrotron x-ray results have researchers rethinking the structure of water.

9 HEALTHY PARTNERSHIPS. A revolution in public health is fundamentally shifting the way medicines are developed and delivered to the world's poorest people. The traditional patchwork of aid givers—foundations, rich countries, various branches of the United Nations, academics, pharmaceutical companies, and charities—have joined forces in myriad joint ventures.

This year, such "public-private partnerships" were behind several headline-making developments, including a promising malaria vaccine trial in Mozambique and the stepped-up efforts to provide anti-HIV drugs to the world's poor. "It's pretty interesting to see how much different it is from 10, 15 years ago," says epidemiologist Roy Widdus, who started the Initiative on Public-Private Partnerships for Health in Geneva, Switzerland. "It really is dramatic."

Widdus dates the movement to the mid-1990s and the formation of the International AIDS Vaccine Initiative (IAVI), which links academics and vaccine manufacturers to develop products for poor countries. His group has identified 91 other health-related public-private partnerships. Roughly 20 of them follow IAVI's lead in developing products that may provide new preventives and treatments for everything from HIV/AIDS, malaria, and tuberculosis to the more obscure tropical diseases. For example, drugmaker Novartis and the Singapore Economic Development Board this year opened the Novartis Institute for Tropical Diseases, which hopes to develop novel drugs for dengue fever and drug-resistant tuberculosis.

Other partnerships aim to improve access to existing medicines. The largest—the Global Fund to Fight AIDS, Tuberculosis, and Malaria—has committed $3 billion to 128 countries since 2002. Widdus estimates that the Bill and Melinda Gates Foundation funds about 75% of the partnerships.

The boom could go bust, however, if the partnerships don't remain accountable, transparent, and productive. "These are 20-year jobs," says Widdus. "Funders and donors change, and they're going to have to reeducate people every couple years and convince them to keep public-private partnerships fashionable items. And if they don't keep funders like Gates going, they're going to be in serious trouble."

10 GENES, GENES EVERYWHERE. It sounds too good to be true: Take water from the ocean or from deep underground, find the DNA in it, sequence the genes, and use them to identify the organisms that live there. Ecologists and evolutionary biologists have tapped such molecular techniques to study the genetic relationships of species they can't grow in the lab. Now ambitious genome sequencers are isolating whole genomes instead of single genes. The genomes provide not only clues about an organism's identity but also a glimpse of how a particular species survives. The work is also turning up thousands of new genes.

One team of biologists sailed across the Sargasso Sea, deciphering genomes from life in 1500 liters of water samples. They turned up more than 1 million new genes. To compensate for the Sargasso's paucity of phosphorus, its denizens had evolved many genes for taking up this mineral. Furthermore, many species are using rhodopsin pigment in lieu of chlorophyll to process carbon. The researchers are now retracing Charles Darwin's voyage on the *Beagle* to explore diversity around the globe.

Another team of environmental genomicists has focused on a small, bizarre microbial community more than a kilometer down, inside an abandoned mine. The organisms thrive without light and instead get their energy by processing iron compounds. DNA in water on the mine floor yielded just five genomes, and the repertoire of enzymes found in each of the five microbes indicated that they had a close relationship, depending on one another to survive in those harsh conditions. With this community's composition in hand, researchers are now tackling a more complex community. They are sampling soil on a farm with the goal of defining the microbial biota there.

Down deep. DNA studies revealed a mine's tight-knit microbial community.

–THE NEWS STAFF

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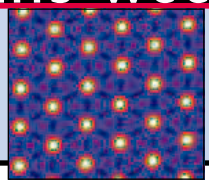
NEWS

This Week



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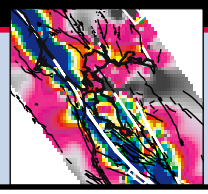


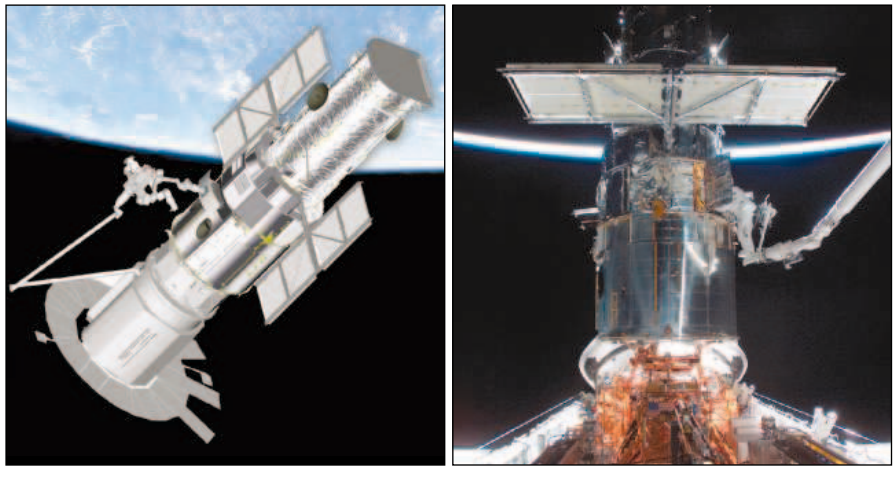
2022
Bracing for the next Big One

NASA

O'Keefe to Go, But Hubble Remains a Battleground

U.S. President George W. Bush wants to put humans back on the moon and, eventually, Mars. To do that, NASA needs to phase out older programs like the space shuttle and the international space station and use the savings for moon-Mars exploration. But one of those dinosaurs—the Hubble Space Telescope—refuses to go quietly.

Last week, a 21-member panel assembled by the National Academy of Sciences told NASA Administrator Sean O'Keefe, in no uncertain terms, that Hubble's life should be extended (www.nap.edu/catalog/11169.html). It also argued that the telescope should be repaired as soon as possible by astronauts aboard the space shuttle rather than by sending a robot, a possibility NASA is currently considering. Accepting the recommendation might have been tough for O'Keefe, given his vocal opposition to the idea. But this week he resigned after 3 years on the job, and his successor, who may be named shortly, may find it easier to embrace the report. Debate over Hubble's future is expected to be the focus of congressional hearings as early as next month.



Two-way fix. Report recommends fixing Hubble with astronauts (*right*) rather than robots (*left*).

Meanwhile, O'Keefe, a former business professor at Syracuse University, is up for the job of chancellor at Louisiana State University in Baton Rouge. In a 13 December letter to the president, O'Keefe says he "will continue until you have named a successor." He said he hoped the Senate would confirm that person by February. A new NASA chief may reverse the agency's current opposition to a shuttle repair mission but will likely still struggle to balance the new exploration effort with established science, shuttle, and station programs.

The academy report results from O'Keefe's decision in February to cancel a fifth shuttle mission to service Hubble. That decision came a year after the Columbia tragedy, which convinced O'Keefe that launching astronauts into an orbit outside that of the international space station, ▶

DEPARTMENT OF ENERGY

Nominee Scores Cabinet Hat Trick

The Bush Administration is revamping its domestic policy lineup with people it knows and trusts (see next page). Few people fit the bill better than Samuel Bodman, who last week was nominated to head the Department of Energy. That's good news for science, say those who have worked with him.

The 66-year-old Bodman has already served nearly 4 years as deputy secretary at the departments of Commerce and Treasury. Trained as a chemical engineer, he's been an associate professor at the Massachusetts Institute of Technology, a venture capitalist, and CEO of Cabot Corp., a Boston-based specialty chemical and energy company. In taking over for Spencer Abraham as energy secretary—his confirmation is seen as a no-brainer for the Senate—Bodman is expected to bring the same straightforward management style that has won him plaudits in his two previous jobs.

Recycler. Samuel Bodman is tapped as Energy Secretary.

"This is a Cabinet secretary who understands what research and innovation is all about because he's lived it," says David Peyton of the National Association of Manufacturers. "His ability to focus on research will depend on outside events, of course, but he knows how to ask the tough questions."

Bodman brings an unusual level of scientific expertise to a post often held by politicians and party loyalists, notes Bruce Mehlman, a consultant who served under Bodman as head of technology policy at the Commerce Department. Mehlman recalls that a trip to the National Institute of Standards and Technology to give a speech was, for Bodman, "like being a kid in a candy store."

Academic leaders also like what they've seen of him. "At Cabot he led with an extraordinary commitment to integrity," says chemist Mark Wrighton, chancellor of Washington University in St. Louis, Missouri, and a director of the $1.8 billion company. "I think the technical leadership within this Administration is dramatically strengthened with this appointment." —JEFFREY MERVIS

CREDITS (TOP LEFT): ADAPTED FROM NASA; (TOP RIGHT) NASA; (BOTTOM) LAWRENCE JACKSON/AP PHOTO

2026
Did the Indus have the write stuff?

2030
Estonia's scientist-diplomat

2031
Time off the tenure clock

which could serve as a safe haven in the event of technical trouble, posed an unacceptable risk. Scrapping the servicing mission condemned Hubble to death by battery and gyroscope failure as early as 2007.

Following an outcry from Congress and scientists, however, O'Keefe agreed to consider a robotic mission instead. Lawmakers then urged creation of an academy panel to review the matter. The panel, chaired by physicist Lou Lanzerotti of the New Jersey Institute of Technology in Newark, spent more than 6 months examining what kind of mission, if any, would make the most sense.

The panel concluded that, first, Hubble is worth saving because of its tremendous contributions to our knowledge of the universe, and, second, that NASA should resurrect its plans to service it with the shuttle "as early as possible after return to flight." "It was clear that the shuttle approach was a much lower mission risk" than sending a robot, says Richard Truly, a former NASA chief who was on the panel. "This is a mission which has been accomplished four times in the past."

The panel also found that the risk to astronauts was not appreciably higher than on a flight to the space station, even though they would have nowhere to go if the shuttle encounters trouble. "If going to the international space station is worth the risk, we believe it is worth the risk to go to Hubble," says panel member Roger Tetrault, who also served on the Columbia accident investigation board. If NASA succeeds in taking care of the technical issues that led to the Columbia failure, "then the need for a safe haven becomes extremely diminished," adds Tetrault. Even so, the panel members noted, a second shuttle could be waiting on the launch pad in case of on-orbit trouble.

A robotic flight, the panel concluded, offers the unsavory mixture of a high technical risk and a low chance of being ready before Hubble's operating systems give out. A separate report done for NASA by the Aerospace Corp. of El Segundo, California, came to a similar conclusion, adding that the cost of a full robotic mission could surpass $2 billion (*Science*, 24 September, p. 1882). The cost of a shuttle mission is hard to pin down, but it could be half that of a robotic flight. It's also more likely to be paid out of NASA's shuttle budget rather than the agency's $4 billion research fund.

Lanzerotti's panel recommended that NASA return to Hubble on the seventh or eighth flight following resumption of operations next summer. Depending on flight rate, that would mean a mission in about 2 years. By then, Truly noted at an 8 December press conference, any bumps in the post-Columbia shuttle system should be ironed out. The new batteries, gyroscopes, and instruments to extend Hubble's life into the next decade have already been built.

The committee's conclusions underscore what many astronomers have been arguing for months. "The case has been clear from the beginning: There are no significant safety issues, and the robotic mission was a pipe dream," says Princeton University astronomer John Bahcall. For Steven Beckwith, director of the Space Telescope Science Institute in Baltimore, Maryland, which operates Hubble, they are further confirmation of the telescope's importance. "Any means by which Hubble can be serviced soon is a great relief to us."

Lawmakers who support Hubble and who backed creation of the academy panel expressed satisfaction with the result. "It's time to fix Hubble; Congress and the American people expect nothing less," said Senator Barbara Mikulski (D–MD), the ranking minority member of NASA's spending panel. Mikulski successfully pushed for $291 million to fund a Hubble servicing mission in the agency's 2005 budget. And Representative Bart Gordon (D–TN) of the House Science Committee said he expected NASA to "heed the academies' assessment and move forward to implement its recommendations so that Hubble can continue its program of scientific exploration and discovery." Both House and Senate lawmakers pledged to hold hearings on the matter early in the new year.

NASA is studying the recommendations, says agency spokesperson Robert "Doc" Mirelson. In the meantime, he says, NASA will continue work on a robotic flight but "will not do anything to preclude a space shuttle mission."

–ANDREW LAWLER

CREDIT: KATSUMI KASAHARA/AP PHOTO

BUSH CABINET

EPA's Leavitt Tapped for Health Post

In an unexpected Cabinet shuffle, the Bush Administration this week nominated Michael Leavitt, head of the Environmental Protection Agency (EPA), to take over the reins at the Department of Health and Human Services (HHS). He will replace Tommy Thompson, who announced his resignation on 3 December.

HHS-bound. EPA's Mike Leavitt stays in town.

The front-runner was thought to be Mark McClellan, a physician and economist who now heads Medicare. But some researchers who know Leavitt are pleased, citing his reputation as a political moderate and supporter of biomedical technology as three-term governor of Utah. "I think he'll be terrific," says Stephen Prescott, executive director of the Huntsman Cancer Institute at the University of Utah in Salt Lake City.

Leavitt spent only 13 months at EPA, succeeding Christine Todd Whitman. Former EPA science chief Paul Gilman says Leavitt insisted on grounding regulations in science, although many environmentalists feel that the agency has been too friendly to industry. But even skeptics agree that EPA has avoided the criticism Thompson faced at HHS for its alleged politicization of science (*Science*, 10 December, p. 1876). At press time the president had not nominated a replacement for Leavitt.

As governor of Utah, Leavitt was a strong proponent of state support for technology to boost the economy. His administration expanded engineering education at universities and helped fund a nonprofit demographic and genetic database on Utah's population. "He was a very big supporter of science with a public health impact," Prescott says.

Leavitt's views on human embryonic stem cells, a likely hot-button issue next year, are not known. That and drug safety reviews at the Food and Drug Administration, which is under his jurisdiction, are likely to be discussed at Leavitt's Senate confirmation hearing.

–JOCELYN KAISER AND ERIK STOKSTAD

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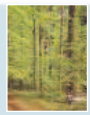
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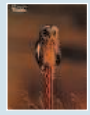
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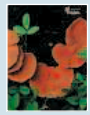
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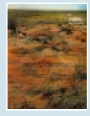
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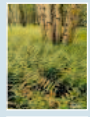
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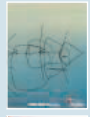
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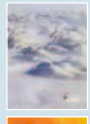
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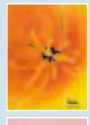
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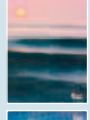
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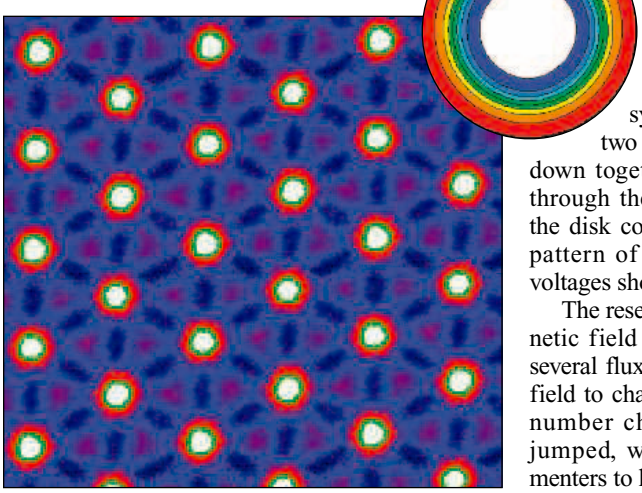
QUANTUM PHYSICS

The Quantum Perfect Storm

In Sebastian Junger's 1997 bestseller *The Perfect Storm*, two storms merge to form a gargantuan cyclone. Now, physicists have spotted the quantum-mechanical equivalent: the merging of several tiny whirlpools of current in a superconductor into a single "giant vortex." Fulfilling a decades-old prediction, the observation may foreshadow stranger things to come and help lay the groundwork for the budding field of "fluxonics."

Researchers have had indirect evidence of the giant vortices (actually less than a micrometer across) and have been striving to image them with sophisticated scanning techniques. But Akinobu Kanda of the University of Tsukuba, Japan, Ben Baelus of the University of Antwerp, Belgium, and colleagues have taken a shortcut to the first direct evidence for the jumbo swirls, as they report in the 17 December *Physical Review Letters*. "It's clever," says Simon Bending, a physicist at the University of Bath, U.K. "In hindsight, I don't know why we didn't do this."



Bull's-eye. Instead of the usual triangular pattern, vortices in a tiny superconducting disk can form a more complicated pattern (*inset*, *top*) or merge into a giant vortex (*inset*, *bottom*).

CREDIT: J. C. SÉAMUS DAVIS/CORNELL UNIVERSITY; (INSETS) BEN BAELUS/UNIVERSITY OF ANTWERP

Whirlpools of current arise when a magnetic field penetrates a superconductor, in which current flows without loss of energy. The magnetic field threads the eyes of the "vortices," and thanks to quantum mechanics, each vortex contains precisely one fundamental quantum of magnetic flux. The vortices repel one another, so they arrange themselves in a triangular pattern. If the superconductor is tiny, however, the cramped vortices should form more exotic patterns and even merge into one jumbo vortex containing several flux quanta, according to the prevailing Ginzburg-Landau theory of superconductivity.

Since the 1990s, physicists had found indirect evidence of the giant vortices by studying the magnetization of a tiny superconducting disk in a varying magnetic field, among other techniques. But they inferred the current distribution from computer simulations. Kanda and colleagues probed the currents directly, by placing two tiny electrodes called "tunnel junctions" on the edge of a 1.5-micrometer-wide aluminum disk 120 degrees apart. They measured the voltage from each junction to a third electrode 120 degrees from each of the other two. The voltages depended on the currents flowing under the tunnel junctions. So if the disk contained a single, symmetrical giant vortex, the two voltages should go up and down together as the magnetic field through the disk changed slightly. If the disk contained a less symmetrical pattern of several vortices, the two voltages should change independently.

The researchers ramped up the magnetic field so that the disk contained several flux quanta and then varied the field to change number. Each time the number changed, the two voltages jumped, which allowed the experimenters to keep the tally as they looked for the subtler signals. In the relatively quiescent times between some jumps, the two voltages went their own ways, indicating several vortices. In between others, the voltages varied in parallel indicating a single vortex. Thus, the researchers demonstrated the merging of individual vortices into one big vortex.

"This evidence is probably 10 times stronger than before," says Andre Geim of the University of Manchester, U.K., who performed the magnetization measurement. Victor Moshchalkov of Catholic University of Leuven in Belgium says the experiment is a step toward observing even stranger vortices, including ones containing fractional flux: "There's a lot of new physics coming up."

In the meantime, Kanda hopes to use the technique to monitor and control the positions of vortices in so-called fluxonic devices. Whereas electronic microchips shuttle electrons, fluxonic chips would shuttle vortices, so that information would literally swirl through them. **–ADRIAN CHO**

ScienceScope

U.S., Kazakhstan Ink Pact for Bioweapons Monitoring

ALMATY, KAZAKHSTAN—A $35 million effort to help fight global bioterrorism moved ahead last week with the signing of an agreement between Kazakhstan and the United States. The initiative—part of the Pentagon's Nunn-Lugar Cooperative Threat Reduction Program—aims to secure dangerous pathogens, guard against the emergence of new strains, and help keep former Soviet bioweapons experts peacefully occupied at facilities that were key cogs in what was once a vast R&D network.

The money will be used in part to create a disease surveillance and diagnostic lab at the Kazakh Science Center for Quarantine and Zoonotic Diseases in Almaty, a former Soviet biodefense institute that tracks endemic plague. Construction is expected to begin in early 2005 and last 2 years. One major challenge for the new lab, says center director Bakyt Atshabar, will be surveillance of a former bioweapons test site on Vozrozhdeniye Island in the Aral Sea.

–RICHARD STONE

Two SARS Vaccines Move Ahead

This week the first of 10 volunteers received a jab with a candidate SARS vaccine as part of a trial at the National Institute of Allergy and Infectious Diseases (NIAID) in Bethesda, Maryland. The vaccine, the second to be tested in humans, consists of a small circular piece of DNA expressing a protein resembling one on the surface of the SARS coronavirus. A study reported in *Nature* last spring demonstrated that the vaccine works in mice; the NIAID trial aims to find out whether it's safe for humans and able to elicit an immune response.

Meanwhile, Sinovac, a biotech company in Beijing, has announced the first results from a similar trial with 36 people using a vaccine produced from killed SARS virus. The study, which has yet to be published, established safety and antibody production, Sinovac said in a 5 December statement. But the company must wait for a new outbreak to test the vaccine's efficacy, says managing director Yin Weidong. Since SARS was brought under control worldwide in July 2003, only a handful of new cases have occurred, most of them as a result of lab accidents.

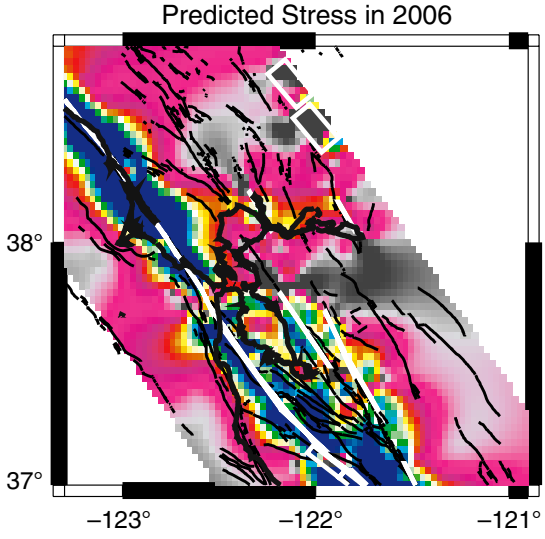
–MARTIN ENSERINK

SEISMOLOGY

Eavesdropping on Faults to Anticipate Their Next Move

Any active earthquake fault talks to its neighbors, urging some to rupture and cautioning restraint among others. The language of faults is stress (*Science*, 22 October 1999, p. 656). The more of it a fault hears, the more likely the fault is to fail, causing an earthquake; take away the stress, and a fault's failure is delayed. Seismologists studying this language of stress have now come out with their most comprehensive attempt to reconstruct past conversations among faults, with an eye toward forecasting where the next moderate to large quakes will strike. Drawing on 160 years of quake history, this latest model builds the most detailed picture yet of present-day crustal stress across the San Francisco Bay area. It's a cautionary picture for residents of the East Bay.

The Bay Area effort "is the first attempt to build a complete model" of evolving crustal stress, says Roland Burgmann of the University of California, Berkeley. "It's an important step and really is the way to go with earthquake hazard forecasting."



Stress quilt. Earthquakes have cast "shadows" of low stress (blue) over the Bay Area, but growing pockets of high stress (gray) remain.

Forecasting stress on faults is something like forecasting the weather using computer models. Both involve Earth systems that evolve over time, given relevant driving forces. In the Bay Area stress model described by seismologists Fred Pollitz, William Bakun, and Marleen Nyst of the U.S. Geological Survey (USGS) in Menlo Park, California, in the 30 November online *Journal of Geophysical Research*, the system is a 100-kilometer-thick block of crust and underlying mantle. It spans the 130-kilometer-wide boundary where the great Pacific tectonic plate is trying to push past North America. The model's chunk of Earth has a San Andreas fault slicing through the upper crust just west of San Francisco, with secondary faults splaying off the San Andreas to the east.

The Menlo Park model also includes the usual processes that determine how high stress gets at any one spot. The two plates move by each other while locked together, deforming the crustal block as if it were so much rubber and steadily loading stress evenly across it. Episodically, earthquakes release and redistribute some of that stress. When a segment of fault ruptures, it relieves stress around the fault—forming a "stress shadow"—but adds stress to the crust beyond the ends of the ruptured segment.

Unlike its predecessors, the Menlo Park model's lower crust and mantle can not only deform as stress changes but also slowly flow, redistributing crustal stress farther afield and weakening a stress shadow faster than in previous models. Pollitz also included 15 earthquakes since 1838, not just the great San Francisco quake of 1906.

With its greater realism, the Menlo Park model painted a fairly accurate picture of stress accumulation, to judge by where quakes struck. All but one of the 22 moderate or large quakes of the past 160 years struck on faults the model indicates were under higher-than-average stress. The 1906 quake started in a high-stress area, according to the model. The huge 1906 stress shadow shrank back across many area faults, which presumably triggered the jump in seismic activity around 1980. And there have been no substantial quakes in the sizable shadow that the model predicts was cast by the 1989 Loma Prieta quake.

In the model's rendition of current stress, two areas of highest stress stand out. Each runs east-west, with its western end overlapping the Rodgers Creek fault north of the bay and the northern Hayward fault (essentially the southern extension of Rodgers Creek) just east of the bay, around densely populated Oakland and Berkeley. In 2002, the Working Group on California Earthquake Probabilities established by the USGS gave the Hayward–Rodgers Creek fault its highest probability for a single fault.

Despite reservations about some details, seismologist Robert Simpson of USGS in Menlo Park (not a co-author of the paper) calls the new stress map "quite an impressive achievement." Such modeling could point to the most likely places for the next quakes, but researchers will still have to do more than eavesdrop if they are going to forecast not just where, but when, the next quake is going to strike. **–RICHARD A. KERR**

BIODEFENSE

Experts Warn Against Censoring Basic Science

CAMBRIDGE, U.K.—Bioterrorism emerged from a potential to a real nightmare 3 years ago when anthrax-laden letters killed five people in the United States. But governments should not respond by screening publications to keep risky-looking information out of terrorists' hands, a new report concludes.* Instead, says the 13 December paper issued jointly by the U.K.'s Royal Society (RS) and the Wellcome Trust, governments should ask scientific societies and funding institutions to take more responsibility for vetting and preventing the dissemination of risky technical details. For example, it suggests that grant review forms could include a check box for bioterror issues to ensure that they are considered.

The recommendations come out of a conference of 66 experts in October organized by the RS and the Wellcome Trust. The participants' "strongly held view," according to the report, is that censoring basic research would not prevent terrorist attacks but could make it more difficult to anticipate and prevent harm. Although many were skeptical of codes of conduct and ethics programs, the group recommended that scientists draw up their own standards for preventing the release of risky data and enforce them. In "very rare cases," the report says, "consideration could be given to delaying publication of highly sensitive information, or releasing only some of the information into the public domain." It does not say how this should be done.

Wellcome Trust director Mark Walport said during the meeting that "we must be seen to have our house in order." He later summarized the consensus: "Self-governance by the scientific community rather than new legislation is the best way forward."

–ELIOT MARSHALL

**Do No Harm: Reducing the potential for the misuse of life science research* (www.royalsoc.ac.uk/news.asp?id=2831).

CREDIT: F. POLLITZ

NEUROSCIENCE

Mutant Gene Tied to Poor Serotonin Production and Depression

Researchers are closely scrutinizing a gene that could explain why some people are depressed—and also why they don't respond to antidepressant drugs that act on the neurotransmitter serotonin. A team headed by cell biologist Marc Caron of Duke University in Durham, North Carolina, has found that a group of severely depressed people were 10 times as likely as nondepressed controls to have a gene variant that reduces the expression of serotonin in the brain.

It's "a very exciting finding, as it represents the first functional [variant] in the key enzyme that synthesizes brain serotonin," says neuroscientist Huda Akil of the University of Michigan, Ann Arbor. "This is exactly what the 'serotonin hypothesis' of depression would have predicted." The study "suggests that we can begin to break major depression into subgroups," adds psychiatrist Thomas Insel, head of the National Institute of Mental Health.

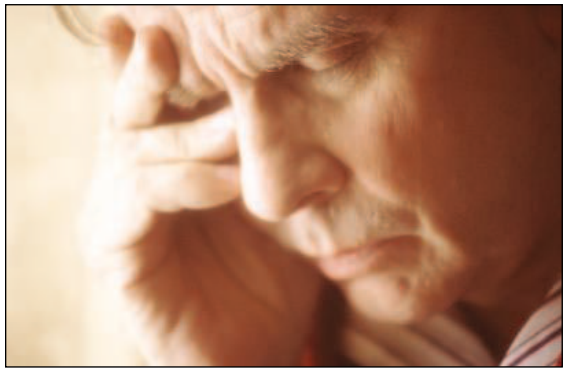
The focus of the new study is the gene for tryptophan hydroxylase-2 (Tph2), an enzyme that controls serotonin production in the brain. The researchers had established in earlier mouse studies that there is a direct connection between Tph2 variation and the rate of serotonin synthesis (*Science*, 9 July, p. 217). More recently, they found that human cells expressing one mutant form of the enzyme produced 80% less serotonin than is made by cells expressing the more common form. In the current study, reported online in *Neuron* on 9 December, Caron's group reveals that in a group of 87 elderly patients with a history of major depression, nine carried the mutated gene variant encoding the poor producer of serotonin, compared with just three in a control group of 219 individuals.

Moreover, even though they weren't diagnosed with depression, the three control subjects with the Tph2 mutation still had problems, such as generalized anxiety, mild depression, or family histories of alcohol abuse or mental illness. The mutation, which changes the enzyme by a single amino acid, appears to be specific to unipolar depression—no one in a group of 60 patients with manic depression, or bipolar disorder, had it.

This is the first gene linked to unipolar depression that has a documented functional effect in brain chemistry, according to Caron. Last year a team headed by Avshalom Caspi of King's College, London, tied vulnerability to depression to a mutant version of a transporter gene that fine-tunes transmission of serotonin (*Science*, 18 July 2003, pp. 291, 386). However, says Caron, that was an association study and not one in which the mutation was clearly shown to affect serotonin in the brain. "That's the exciting thing about our mutation," explains Caron. "We have been able to document in a biochemical way that it does affect function."

Caron and his colleagues suggest that the mutation could help predict who will be helped by selective serotonin reuptake inhibitors (SSRIs) such as Prozac. Seven of the depressed subjects with the mutant Tph2 allele failed to respond to SSRIs, and the other two required extremely high doses. Apparently, patients with the mutation put out so little serotonin that SSRIs, which cause the chemical to linger in a synapse, make little difference.

What's more, citing unpublished mouse studies, Caron hints that the mutation could play a role in some of the problems associated with SSRI use, including extreme agitation, psychosis, and suicidal behavior. Such reactions have caused both the United Kingdom and the United States to issue warnings about prescribing SSRIs to children and adolescents.



Running low. A mutant gene that decreases serotonin production may spur depression and stymie antidepressants.

Depression is likely influenced by many different genes, but if future, larger studies support the importance of Tph2 variants, says Akil, "it would represent a real breakthrough" that could help clinicians detect susceptibility to depression as well as tailor drug treatment to a patient's genetic profile. Says Insel: "This is just the first paragraph in what will be a long and fascinating new chapter about serotonin and depression."

–CONSTANCE HOLDEN

CREDIT: JOSE LUIS PELAEZ INC./CORBIS

ScienceScope

School Board Sued Over "ID"

This week the parents of 11 Pennsylvania students sued their local school officials for requiring children to learn "other theories of evolution including ... intelligent design (ID)" (*Science*, 5 November, p. 971). The suit, filed with the help of the American Civil Liberties Union, says that the policy, adopted this fall by the Dover (Pennsylvania) school board, violates their religious liberty.

The school board policy is widely seen as violating a 1987 Supreme Court ruling on the separation of church and state, one that creationists have tried to sidestep by focusing on so-called scientific objections to Darwinism. Even the Discovery Institute of Seattle, Washington, the movement's think tank, says the Dover policy is muddled and "raises serious problems from the standpoint of constitutional law."

–CONSTANCE HOLDEN

USDA Eyes Plant Imports

The U.S. Department of Agriculture (USDA) has proposed tightening regulation of imported live plants—a major vector for pests and invasive weeds. Except for a limited blacklist, any plant can currently be imported if it is inspected before export and checked for disease upon arrival. But USDA's Animal and Plant Health Inspection Services (APHIS) worries that better safeguards are needed. The toughest option that APHIS proposes in the 10 December *Federal Register* is to restrict large shipments of some plants until the agency is sure they will not spread pests or become troublesome weeds.

"The potential is to greatly improve protection against invasive species," says Richard Orr of the interagency National Invasive Species Council in Washington, D.C. Comments are due by 10 March.

–ERIK STOKSTAD

ACS Sues Google

Imitation may be the sincerest form of flattery, but the American Chemical Society (ACS) isn't pleased with Google Scholar, an academic research tool that ACS says is too similar in name and function to Scifinder Scholar, the society's own search service.

The society's suit, filed 9 December in federal court, claims that Google has infringed on ACS's trademark and is competing unfairly. ACS wants Google to immediately change the name and pay unspecified damages. Google spokesperson Steve Langdon says the company is "confident" in its use of the chosen name.

–YUDHIJIT BHATTACHARJEE

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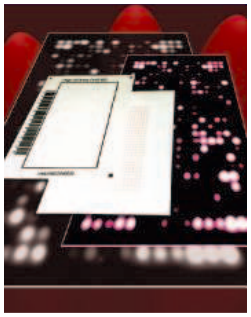

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JAPANESE UNIVERSITIES

Junior Faculty Hope Name Change Will Lead to Greater Independence

TOKYO—Sometimes, it's hard to distinguish an assistant professor at a Japanese university from a professor's assistant. By tradition and law, Japanese academic departments are broken up into *koza* (chairs), in which a full professor oversees one or two assistant professors as well as lecturers and research associates. The professor will often pick the assistant's research topics—and take credit for the results—or, conversely, fill their schedule with teaching duties.

But change is coming. Last month, a Ministry of Education advisory committee recommended scrapping the *koza* system. Assistant professors would become associate professors with the same educational and research duties as professors but at a lower rank. Lecturers and research associates would also receive greater independence.

The *koza* scheme, borrowed in the mid-1800s from the German academic model, "has gotten out of date," says Yasuhiko Torii, an economist and former president of Keio University, who heads the advisory committee. "Sometimes younger scientists have no research freedom." The *koza* structure and the status of assistant professors and lecturers are defined by several laws that the committee wants amended.

Some academics welcome the recommendations. "It's a change for the better," says Kumiko Ogoshi, a research associate in environmental health at Nara Medical University, who in 2002 won $1100 from her university after suing her professor for "academic harassment." But the real test, she says, will be seeing who actually makes the decisions on promotions and assignments. "If [such decisions] are still up to a single professor, the recommendations should be reconsidered," she says.

Team effort. Gifu University's Kenichi Tezuka says he already shares responsibility with his chair.

Departments at many leading universities have already abandoned the *koza* system and strengthened the hand of younger scientists. "In my case, I independently conduct my own research," says Kenichi Tezuka, an assistant professor specializing in bone biology at the Graduate School of Medicine of Gifu University in Gifu City. He and his professor split the *koza*'s teaching and administrative duties, he adds.

The new system will need to retain some flexibility to account for the differences among disciplines and universities, says advisory committee member Reiko Kuroda, a professor of biochemistry at the University of Tokyo and a member of the advisory committee. She feels that a more clearly defined status for associate professors should also foster competition—and thus strengthen the research enterprise—by making it easier for academics to move to a new institution.

The committee is soliciting comments and hopes to finalize its recommendations next spring. They would go into effect in 2006 or 2007. **–HIROMI YOKOYAMA**

Hiromi Yokoyama is a freelance science writer in Tokyo.

INFLUENZA

WHO Adds More "1918" to Pandemic Predictions

Call it a crash course in the vagaries of risk communication. Until now, the World Health Organization (WHO) has been deliberately cautious in estimating how many people a new influenza pandemic might kill. Dire projections, WHO officials have worried, could damage its credibility. But last week, the agency bowed to experts—including one from its own ranks—who have been ratcheting up the projected death toll in recent months. WHO conceded in a statement that scientifically valid assumptions range as high as 50 million or more—at least seven times WHO's previous maximum number.

How deadly a pandemic will be depends on many factors: for instance, the pathogenicity of the new virus strain, the speed at which it spreads, and how much vaccine is available. Although the specter of millions of deaths might help inject a sense of urgency into the worldwide campaign to prepare, says WHO flu chief Klaus Stöhr, such estimates may also erode trust if the numbers prove too high, or if the pandemic fails to materialize within the next few years.

Doing the math. Klaus Stöhr prefers cautious flu death toll estimates.

That's why WHO stuck to a conservative message, Stöhr says. On its Web site, it cited data from the U.S. Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, showing that "today, a pandemic is likely to result in 2 to 7.4 million deaths globally." The numbers were produced by CDC health economist Martin Meltzer, who used a computer model based on a virus strain similar to the one that caused a mild pandemic in 1968.

But others say the next pandemic strain may just as well be highly virulent, like the one that caused the 1918–19 Spanish flu, which claimed at least 20 million lives and perhaps many more. WHO's earlier numbers are "rather ridiculous," says Michael Osterholm, director of the University of Minnesota Center for Infectious Disease Research and Policy in Minneapolis. In a 25 November e-mail to Stöhr, Osterholm pointed out that given today's world population, a 1918-like virus could kill at least 72 million. "World leaders need to get this message," Osterholm says. On 29 November, a similar message was sounded by Shigeru Omi, director of WHO's Western Pacific Region Office in Manila, who broke ranks by saying publicly that the toll could be as high as 20 million, 50 million, or "in the worst case," 100 million.

Initially, WHO had hoped to end the debate by coming up with new, science-based numbers itself. But there's simply too little anyone can say with any certainty, according to WHO spokesperson Richard Thompson. So a carefully worded statement issued on 8 December and approved at the highest levels simply concludes that experts' estimates "have ranged from 2 million to over 50 million. All these answers are scientifically grounded." The statement calls the earlier 2-to-7-million estimates "best-case scenarios."

The new statement is "still lacking in leadership," Osterholm says. But Peter Sandman, a risk communications consultant from Princeton, New Jersey, who has advised WHO, says the new statement is "a huge improvement" because it acknowledges the scientific uncertainty rather than favoring one scenario. **–MARTIN ENSERINK**

CREDITS (TOP TO BOTTOM): D. NORMILE; WHO

For 130 years scholars have struggled to decipher the Indus script. Now, in a proposal with broad academic and political implications, a brash outsider claims that such efforts are doomed to failure because the Indus symbols are not writing

The Indus Script—Write or Wrong?

Academic prizes typically are designed to confer prestige. But the latest proposed award, a $10,000 check for finding a lengthy inscription from the ancient Indus civilization, is intended to goad rather than honor. The controversial scholar who announced the prize last month cheekily predicts that he will never have to pay up. Going against a century of scholarship, he and a growing number of linguists and archaeologists assert that the Indus people—unlike their Egyptian and Mesopotamian contemporaries 4000 years ago—could not write.

That claim is part of a bitter clash among academics, as well as between Western scientists and Indian nationalists, over the nature of the Indus society, a clash that has led to shouting matches and death threats. But the provocative proposal, summed up in a paper published online last week, is winning adherents within the small community of Indus scholars who say it is time to rethink an enigmatic society that spanned a vast area in today's Pakistan, India, and Afghanistan—the largest civilization of its day.

Searching for script. Richard Meadow excavates at Harappa.

The Indus civilization has intrigued and puzzled researchers for more than 130 years, with their sophisticated sewers, huge numbers of wells, and a notable lack of monumental architecture or other signs of an elite class (see sidebar on p. 2027). Most intriguing of all is the mysterious system of symbols, left on small tablets, pots, and stamp seals. But without translations into a known script—the "Rosetta stones" that led to the decipherment of Egyptian hieroglyphics and Sumerian cuneiform in the 19th century—hundreds of attempts to understand the symbols have so far failed. And what language the system might have expressed—such as a Dravidian language similar to tongues of today's southern India, or a Vedic language of northern India—is also a hot topic. This is no dry discussion: Powerful Indian nationalists of the Hindutva movement see the Indus civilization as the direct ancestor to Hindu tradition and Vedic culture.

Now academic outsider Steve Farmer (see sidebar on p. 2028) and two established Indus scholars argue that the signs are not writing at all but rather a collection of religious-political symbols that held together a diverse and multilingual society. The brevity of most inscriptions, the relative frequencies of symbols, and the lack of archaeological evidence of a manuscript tradition add up to a sign system that does not encode language, argue historian Farmer and his co-authors, Harvard University linguist Michael Witzel and computational theorist Richard Sproat of the University of Illinois, Urbana-Champaign. Instead, they say the signs may have more in common with European medieval heraldry, the Christian cross, or a bevy of magical symbols used by prehistoric peoples.

This idea has profound implications for how the Indus civilization lived and died. Instead of the monolithic, peaceful, and centralized empire envisioned by some scholars, the authors say that the new view points to a giant multilingual society in which a system of religious-political signs provided cohesion.

Their thesis has bitterly divided the field of Indus studies, made up of a small and close-knit bunch dominated by Americans. Some respected archaeologists and linguists flatly reject it. "I categorically disagree that the script does not reflect a language," says archaeologist J. Mark Kenoyer of the University of Wisconsin, Madison, who co-directs a dig at the key site of Harappa in Pakistan. "What the heck were they doing if not encoding language?" Asko Parpola, a linguist at Finland's University of Helsinki who has worked for decades to decipher the signs, says. "There is no chance it is not a script; this is a fully formed system. It was a phonetic script." Linguist Gregory Possehl of the University of Pennsylvania in Philadelphia says that it is not possible to "prove" the script cannot be deciphered. All three argue that Farmer's thesis is a pessimistic and defeatist approach to a challenging problem. Meanwhile, the very idea that the Indus civilization was not literate is deeply offensive to many Indian nationalists.

Yet since a 2002 meeting at Harvard University at which Farmer laid out a detailed theory—and was greeted with shouts of derision—he has attracted important converts, including his co-authors. A growing cadre of scholars back the authors' approach as a fresh way to look at a vexing problem and an opportunity to shed new light on many of the mysteries that haunt Indus research. Harvard anthropologist Richard Meadow, who with Kenoyer directs the Harappa project, calls the paper "an extremely valuable contribution" that could cut the Gordian knot bedeviling the field. Sanskrit and South Asian linguist Witzel says he was shocked when he first heard Farmer's contention in 2001. "I thought I could read a few of the signs,"

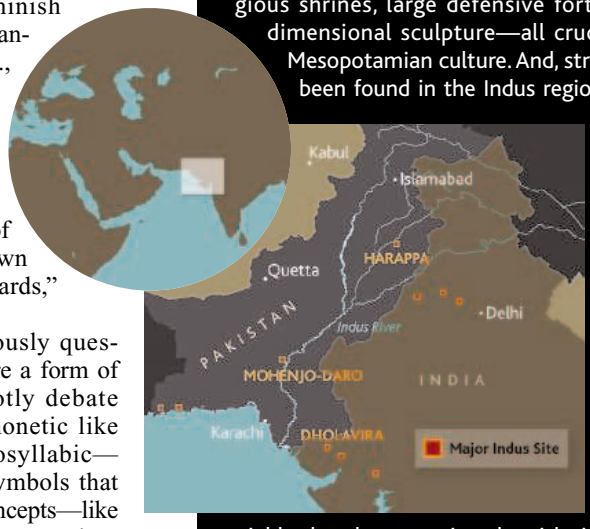
CREDITS: COURTESY OF THE HARAPPA ARCHAEOLOGICAL RESEARCH PROJECT/PHOTOS BY R. H. MEADOW

Witzel recalls. "So I was very skeptical." Now he is throwing his scholarly weight behind the new thesis, as a co-author of the paper and also editor of the *Electronic Journal of Vedic Studies*, an online journal aimed at rapid publication, which published the paper. Adds archaeologist Steven Weber of Washington State University in Vancouver: "Sometimes it takes someone from the outside to ask the really basic questions." Weber, who is now collaborating with Farmer, adds that "the burden of proof now has to be on the people who say it is writing."

Seeking the Write Stuff

Since the 1870s, archaeologists have uncovered more than 4000 Indus inscriptions on a variety of media. Rudimentary signs appear around 3200 B.C.E.—the same era in which hieroglyphics and cuneiform began to appear in Egypt and Iraq. By 2800 B.C.E., the signs become more durable, continuing in use in later periods; the greatest diversity starts to appear around 2400 B.C.E. Some signs are highly abstract, whereas others seem to have obvious pictographic qualities, such as one that looks like a fish and another that resembles a jar. Both are used frequently; the jar sign accounts for one in 10 symbols, says Possehl. As in Mesopotamia, the signs typically appear on small tablets made of clay as well as on stamp seals. The seals often are accompanied by images of animals and plants, both real and mythical.

The signs start to diminish around 1900 B.C.E. and vanish entirely by 1700 B.C.E., when the Indus culture disappears. Oddly, the inscriptions are almost all found in trash dumps rather than in graves or in primary contexts such as the floor of a home. "They were thrown away like expired credit cards," says Meadow.



No one had ever seriously questioned whether the signs are a form of writing. But scholars hotly debate whether the system is phonetic like English or Greek or logosyllabic—using a combination of symbols that encode both sound and concepts—like cuneiform or hieroglyphics. Even the number of signs is controversial. Archaeologist and linguist S. R. Rao of India's University of Goa has proposed a sign list of only 20, but Harvard graduate student Bryan Wells is compiling a revised list now numbering 700; most estimates hover in the 400 range.

Farmer and colleagues reanalyzed the signs, drawing on published data from many sites and unpublished data from the Harappa project provided by Meadow. They found that the average Indus inscription, out of a

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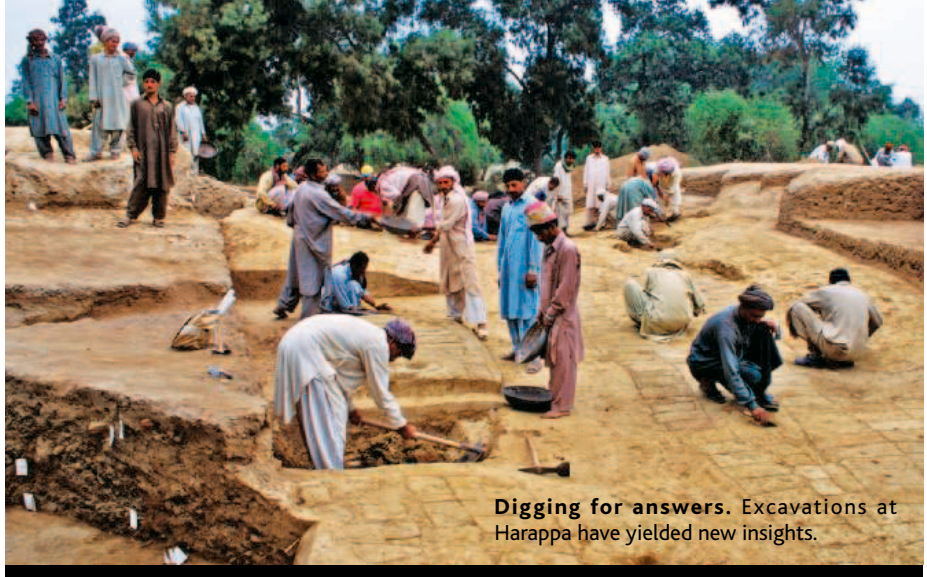

Digging for answers. Excavations at Harappa have yielded new insights.

Splendid Sewers, But Little Sculpture

British explorers stumbled on Indus ruins and artifacts in the late 1870s, but it was not until the 1920s that excavations revealed the geographically largest ancient urban civilization of the 3rd millennium B.C.E. Digs at sites such as Harappa and Mohenjo Daro in Pakistan revealed sprawling cities; Harappa may have been home to 50,000 people in its heyday between 2500 B.C.E. and 2000 B.C.E. Standardized bricks and weights were used in towns and cities more than 1000 kilometers from the civilization's center along the Indus River, and wheeled carts were widespread. The sanitation systems, including extensive wells and underground pipes, were of a sophistication not seen again until 2000 years later in ancient Rome.

The Indus seemed to resemble closely the complexity of riverine societies like those of Egypt and Mesopotamia during the 3rd millennium B.C.E., and the three civilizations apparently had contact. Carnelian and lapis lazuli from the West Asian region made its way to Egypt, and Indus stamp seals have been found in Mesopotamia.

Yet in other ways, the Indus stands alone. It lacks monumental buildings, obvious religious shrines, large defensive fortifications, clear social stratification, and three-dimensional sculpture—all crucial elements of contemporaneous Egyptian and Mesopotamian culture. And, strangely, no Egyptian or Mesopotamian artifacts have been found in the Indus region. The Indus seems isolated and insulated until the turn of the millennium, when the strong influence of cultures to the immediate west became noticeable. By 1700 B.C.E., most traces of Indus material culture vanish suddenly, for no obvious reason and leaving no clear cultural heirs. "For a long time, people thought the Indus was so enigmatic, so unique, that there was no point in comparisons because none of them fit," says Rita Wright, a New York University archaeologist who has worked at Harappa.

That view of the Indus as odd has begun to fade with the most recent series of digs in the ancient city of Harappa, which halted after the events of 9/11. There, and at several sites in India, archaeologists have found evidence of walled neighborhoods suggesting clannish rivalries or outside threats, jewelry of different quality suggesting social distinctions, and civic structures. New digs within India have uncovered evidence of a more vibrant system of trading over long distances. Those finds hint at a society not so radically different from its contemporaries, says Wright. In that light, a thesis highlighting the oddity of the Indus symbols (see main text) feels like a backward step, she adds.

Unraveling the contradictions of the Indus civilization will require more data—data that are buried in the mostly unpublished notes of the Harappa team and their Indian colleagues, at sites along the tense India-Pakistan border, and in tribal areas closed now to scientists. The Indus seems destined to confound archaeologists for decades to come.

–A.L.

Outsider Revels in Breaking Academic Taboos

Steve Farmer describes himself as "the ultimate collaborationist," but he has a way of making enemies. When he showed up at a 2002 Harvard University gathering to propose that the Indus script is no script at all, participants recall that his ideas were greeted with shouts of derision. And his positions on the role of the Indus civilization in Indian history have earned him a place in the demonology of Indian nationalists.

Yet despite what many call an abrasive personality, this former street kid from Chicago, who lacks a high school diploma, has shaken up the closed field of Indus studies (see main text). "It is healthy the way this is turning things upside down," says archaeologist Steven Weber of Washington State University in Vancouver.

Farmer's linguistic ability got him off the streets when he joined the Army in the 1960s. After learning Russian at the military's language school in Monterey, California, he worked for the National Security Agency listening in on the conversations of Soviet pilots. Then, radicalized by the Vietnam War, he left the military for academia. After winning a high school equivalency diploma, he studied history at the University of Maryland, College Park, and earned a Ph.D. in comparative cultural history at Stanford University in California. He taught history of science and European history at George Mason University outside Washington, D.C., and then moved to Louisiana State University in Baton Rouge as a tenure-track professor. But he says he rejected full-time academic life to avoid teaching courses he found boring and moved back to California, where he was on the adjunct faculty at Ohlone College in Fremont until 1997. To support his scholarly pursuits, Farmer has edited a journal on narcolepsy, worked on a PGA golf tournament training program, and helped develop a device to aid people with brain disorders.

Indus iconoclast. Steve Farmer holds a replica of the longest Indus inscription.

In 1999, after putting together a model of cross-cultural frameworks for premodern history using ancient China as an example, he turned his attention to India. "I didn't know anything about this stuff," he says. "I was the naïve outsider too dumb not to recognize the field's taboos." But he was struck by the brevity of Indus inscriptions and unconvinced by the many efforts to decipher the symbols. He didn't hesitate to poke fun at Indian nationalists who attempted their own decipherments and who promulgated theories connecting the Indus to Hindu culture. "I still get death threats daily," he says. "And I'm careful about opening packages from India." He also was irritated by what he calls archaeologists' proclivity to "hoard data."

"He can be abrasive and aggressive, and many in the field find him presumptuous," says linguist George Thompson of Montserrat College of Art in Beverly, Massachusetts. At the 2002 Harvard meeting, a few of the academics present hooted Farmer off the stage. "People were literally screaming," Farmer recalls. Nonetheless, his arguments ultimately impressed Harvard anthropologist Richard Meadow, who granted him access to unpublished Harappa data. "Steve stepped in and did an enormous amount of work" on the Harappa data, says Thompson.

His arrogance makes him hard for some scholars to get along with. "I've remade the field," he recently boasted. Others resent his methods. "He uses verbose arguments," says archaeologist J. Mark Kenoyer of the University of Wisconsin, Madison, co-director of the Harappa dig. "And he's not basing it on science." Adds linguist Gregory Possehl of the University of Pennsylvania in Philadelphia, "I don't think his ideas are interesting or viable, and I'm surprised they have raised interest." At this point, however, that interest is undeniable, so Indus specialists are making room, albeit reluctantly, for a new member of their small family. But the intellectually peripatetic Farmer insists he will not make himself at home: "This is just a chapter in my book."

–A.L.

total of 4000 to 5000 in a 1977 compilation, has 4.6 signs. The longest known inscription contains 17 signs, and fewer than 1% are as long as 10 symbols. The authors argued that such short "texts" are unprecedented for actual writing. Although many scholars assert that longer inscriptions may have been made on perishable materials, the authors note that there is no archaeological evidence of the imperishable paraphernalia that typically accompanies literate culture, such as inkpots, rock inscriptions, or papermaking devices.

Farmer and colleagues also take apart a long-held assumption that the frequent repetition of a small number of Indus signs is evidence of a script encoding language. About 12% of an average English text, for example, consists of the letter "E," often used repeatedly in a single sentence to express a certain sound. In contrast, the paper notes that very few Indus symbols are repeated within individual inscriptions, implying that the signs do not encode sounds.

Further, the authors note that many Indus symbols are incredibly rare. Half of the symbols appear only once, based on Wells's catalog; three-quarters of the signs appear five times or fewer. According to the 1977 compilation put together by Iravatham Mahadevan, an Indian linguist now retired in Chennai, India, more than one-fourth of all signs appear only once, and more than half show up five times or fewer. Rarely used signs likely would not encode sound, says Farmer. It is as if many symbols "were invented on the fly, only to be abandoned after being used once or a handful of times," he, Witzel, and Sproat write.

Farmer believes that the symbols have nonlinguistic meaning. He speculates that the signs may have been considered magical—as the Christian cross can be—and indicated individuals or clans, cities or professions, or gods. He and his colleagues compare the Indus script to inscriptions found in prehistoric southeastern Europe around 4000 B.C.E., where the Vinča culture produced an array of symbols often displayed in a linear form, including a handful used frequently.

Short and sweet. Most Indus inscriptions are short.

But these conclusions are not accepted by key archaeologists and linguists who have spent their careers digging at Harappa or trying to decipher the symbols. "Regularities in the frequency and distribution of signs are possible only in a linguistic script," says Mahadevan. Wells is more blunt. "He is utterly wrong," he says of Farmer. "There is

CREDITS (TOP TO BOTTOM): COURTESY OF S. FARMER; COURTESY OF THE HARAPPA ARCHAEOLOGICAL RESEARCH PROJECT/PHOTOS BY R. H. MEADOW

something you recognize as an epigrapher immediately, such as long linear patterns."

As to the brevity of inscriptions, Wells says averages can be misleading. The longer Indus inscriptions, he says, can't be explained as magical symbols. Vinča symbols, for example, rarely are grouped in numbers greater than five. "And you don't get repetitive ordering" as with Indus signs, he adds. "The Indus script is a highly patterned, highly ordered system with a syntax—it just looks too much like writing." Wells also says that a mere 30 signs are used only once, rather than the 1000 Farmer postulates, because many of the "singletons" transform into compound signs used repeatedly.

Parpola agrees that the pattern of symbols argues for an organized script. "There are a limited number of standardized signs, some repeated hundreds of times—with the same shape, recurring combinations, and regular lines," he says. But Wells and Parpola, like most linguists in the field, agree on little beyond their opposition to Farmer. Wells rejects Parpola's method of deciphering the signs, and Parpola dismisses Wells's contention that there are significant differences between the signs of upper and lower Indus.

Wells and some other scholars believe that the attraction of Farmer's idea has less to do with science than with the long history of decipherment failures. "Some have turned to this idea that it is not writing out of frustration," he says.

Sign or script? Farmer says Indus seals (left), like Vinča signs (right) are not writing.

Literacy promoter. J. Mark Kenoyer, on the dig at Harappa, thinks Indus signs are script.

But many others are convinced that Farmer, Witzel, and Sproat have found a way to move away from sterile discussions of decipherment, and they find few flaws in their arguments. "They have settled the issue for me," says George Thompson, a Sanskrit scholar at Montserrat College of Art in Beverly, Massachusetts. "We have the work of a comparative historian, a computational linguist, and a Vedicist," he adds. "Together they have changed the landscape regarding the whole question." In a forthcoming book on South Asian linguistic archaeology, Frank Southworth of the University of Pennsylvania calls the paper an "unexpected solution" to the old troubles with decipherment.

Meanwhile, Farmer is injecting a bit of fun into the melee. "Find us just one inscription with 50 symbols on it, in repeating symbols in the kinds of quasi-random patterns associated with true scripts, and we'll consider our model falsified," he wrote on a listserve devoted to the Indus. And he is putting his money—or, rather, that of a donor he won't reveal—where his mouth is, promising the winner $10,000. The orthodox dismiss the prize as grandstanding, whereas Farmer boasts that "no one is ever going to collect that money."

Retrenching

Each side clearly has far to go to convince its opponents. "I'm not sure the case is strong enough on either side," says linguist Hans Hock of the University of Illinois, Urbana-Champaign. "Let each side of the controversy make their case."

Yet there already is a retreat from earlier claims that the Indus symbols represent a full-blown writing system and that they encoded speech. Many scholars such as Possehl now acknowledge that the signs likely are dominated by names of places, people, clans, plants, and gods rather than by the narratives found in ancient Sumer or Egypt. They say the script may be more similar to the first stages of writing in those lands. Harvard archaeologist Carl Lamberg-Karlovsky says the meanings of the Indus signs likely are "impenetrable and imponderable" and adds that whether or not the signs are considered writing, they clearly are a form of communication—and that is what really counts. Recent research in Central and South America has highlighted how complex societies prospered without traditional writing, such as the knotted strings or khipu of the vast Incan empire (*Science*, 2 July, p. 30).

Farmer adds that a society does not need to be literate to be complex. "A big, urban civilization can be held together without writing," he says. He and his co-authors suggest that the Indus likely had many tongues and was a rich mix of ethnicities like India today. Wells has found marked differences between signs in the upper and lower Indus River regions, backing up the theory of a more diverse society. But some, such as D. P. Agrawal, an independent archaeologist based in Almora, India, doubt that a civilization spread over more than 1 million square kilometers, and with uniform weights, measures, and developed trade, could manage its affairs without a script.

This debate over Indus literacy has political as well as academic consequences. "This will be seen as an attack on the greatness of Indian civilization—which would be unfortunate," says Shereen Ratnagar, a retired archaeologist who taught at Delhi's Nehru University. Tension is already high between some Western and Indian scholars and Indian nationalists. "Indologists are at war with the Hindutva polemicists," says statistical linguist Lars Martin Fosse of the University of Oslo, and the issue of the script "is extremely sensitive." Farmer says he regularly receives e-mail viruses and death threats from Indian nationalists who oppose his views.

For decades, Indus researchers have tended to stick with their established positions, as on the script, a tendency that has kept the field from moving forward, says one archaeologist who compares the small cadre of Indus scholars to a "dysfunctional family" with a proclivity for secrecy, ideological positions, and intolerance. Meadow is among those who argue that it is time to set aside old ideas, no matter how much time and effort has been invested in them, in order to push the field forward. "We're here to do science, and it is always valuable to have new models," he says. Adds Ratnagar: "We must get back to an open mind." Given the strong emotions swirling around the Indus symbols, discovering the key to that open mind may prove the hardest code to break.

–ANDREW LAWLER

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Cutting a Path in Genetics and International Diplomacy

The newly elected president of the Estonian Academy of Sciences has long been a scientist-diplomat, first in dealings with the Soviet Union, now with the European Union

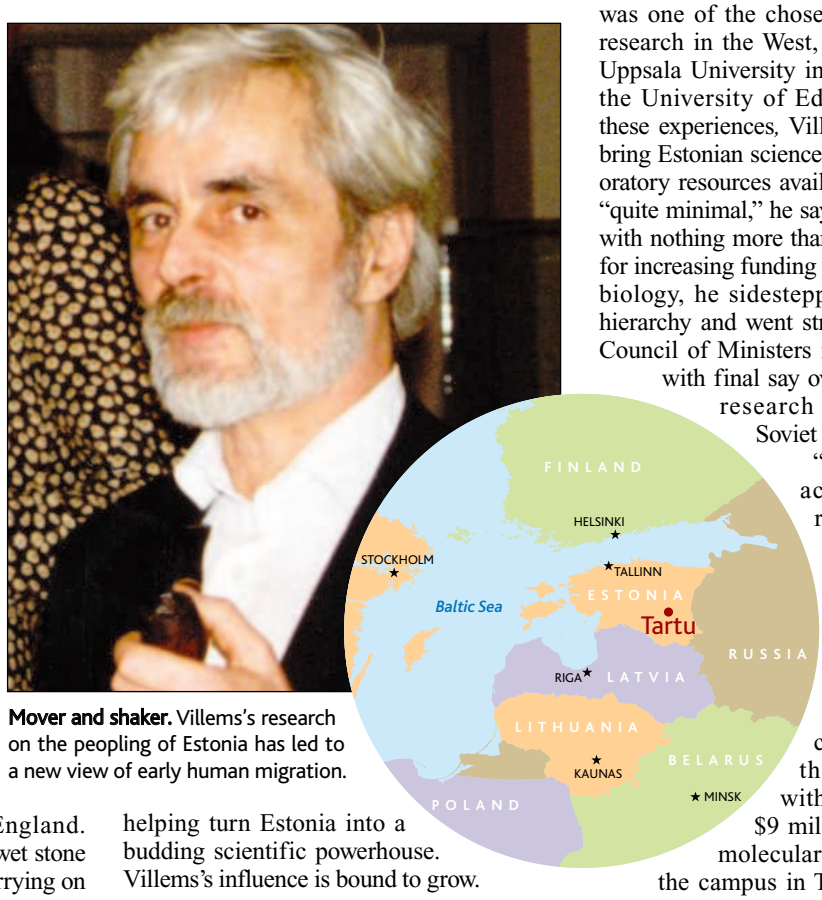
TARTU, ESTONIA—Rather than take the long route to the entrance of his building, Richard Villems leads a visitor through the trees at the back door. "The student way," he says, hopping over a ripped section of the metal fence. At 60, the silver-haired geneticist seems to have lost none of his agility. Known for his research on early human migrations, which is currently challenging some long-held views of the peopling of the world, Villems has played another, public role as well—helping build a research infrastructure in his native Estonia and lending vigor to an academic world that was until recently beholden to bureaucrats in Moscow.

Villems, who was elected president of the Estonian Academy of Sciences last month, nods at a squat concrete building: "That's where I lived for 4 years when I was a medical student," he says with a smile. Back then, "you had to be careful what you said," because people could be expelled from the university or even arrested for politically incorrect behavior.

Things have changed here. This could be the campus of an Ivy League university in New England. Graduate students amble along the wet stone paths carpeted by autumn leaves, carrying on discussions in half a dozen languages. Villems chats among them casually and puffs on his ever-present pipe. And like a well-established Ivy League professor, Villems excuses himself to deal with the paperwork for several multimillion-dollar research grants.

Russian soldiers pulled out of Estonia only 10 years ago; the nation quickly reoriented itself toward the West and was granted membership in the European Union (E.U.) just this May. While most other former communist central and eastern European nations are struggling with poverty and a drain of expertise to richer neighbors, Estonia has emerged from the former Soviet Union's dominion as an economic and academic success story.

Unusual among the new democracies, Estonia's transformation has been spearheaded in large part by its scientists, says Ene Ergma, an astrophysicist who is now the leading politician in the Estonian parliament. She credits Villems as a "science diplomat," helping turn Estonia into a budding scientific powerhouse. Villems's influence is bound to grow.



Mover and shaker. Villems's research on the peopling of Estonia has led to a new view of early human migration.

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Follow the DNA

The Estonians enjoyed a "special status" that allowed a somewhat more relaxed intellectual life than that of others within the Soviet empire, says Villems. One reason, he says, is that the Estonians have always been a breed apart. Their language, like Finnish and Hungarian, comes from a root unrelated to the languages spoken in the rest of Europe. Along with their linguistic oddity comes the riddle of their genetic origins. The prevailing theory once held that the Estonians arrived in a single migration from the Ural mountains in Siberia, but it has been supplanted in the last decade by a more complex theory that the population is a mix of tribes that migrated from several directions.

Villems began to puzzle over this question in the late 1980s. His new passion was opportune. Techniques were just emerging that allow researchers to reconstruct the human family tree using DNA sequences, tracing the split and migration of different populations right back to the appearance of *Homo sapiens* in Africa more than 100,000 years ago. And Villems had by then gained the prestige and independence to choose and pursue his own project.

Villems had been a rising star among Soviet molecular biologists. In the 1970s he was one of the chosen few allowed to do research in the West, first as a postdoc at Uppsala University in Sweden and then at the University of Edinburgh, U.K. After these experiences, Villems resolved to help bring Estonian science up to speed. The laboratory resources available at the time were "quite minimal," he says. So in 1984, armed with nothing more than a 13-page argument for increasing funding for modern molecular biology, he sidestepped the bureaucratic hierarchy and went straight to the U.S.S.R. Council of Ministers in Moscow, the body with final say over the distribution of research funding within the Soviet Union.

"That was a brilliant act of diplomacy," recalls Jaak Järv, a chemist at the University of Tartu. "Almost no one knew how to deal with such a huge bureaucracy," but Villems pulled it off. The committee rewarded the upstart Estonian with the equivalent of a $9 million grant to create a molecular biology institute on the campus in Tartu, now called the Estonian Biocenter. This was "more than the total that all Estonian scientists had ever received in grants," says Villems. That sum has since been multiplied many times over by private donors and research charities wooed by Villems and others, particularly after the E.U. designated the Biocenter as one of its 34 "Centers of Excellence" in 1999.

Armed with the modern tools of biology, Villems attempted to trace Estonians' origins through the DNA of the mitochondria, which is passed down from mother to child, and the Y chromosome, which passes from father to son. In principle, by comparing the mutations that accumulate in these gender-linked indexes, the age and origin of modern populations can be worked out. It's well established, for example, that all modern

humans trace their parentage to a female line that emerged from Africa more than 100,000 years ago. But sorting out individual European populations is a big challenge. There has been so much mixing among the original tribes over history, says Villems, that "to get the real answers you have to go deeper in time, farther out in the context" than the peopling of just Europe.

Sleuthing the Y-chromosomal DNA of Estonians, for example, seems to lead back to ancient populations from Borneo and the Sunda Islands that spread up to eastern Siberia before the last Ice Age. But if this turns out to be true, says Villems, "it will be beyond any present-day 'standard scenario' of gene flow over the past 20,000 years." Getting the answers is only possible by placing Estonians within "the big picture." And to piece together that picture, Villems has amassed an "amazing" collection of European and Asian DNA samples, says Thomas Gilbert, a British molecular anthropologist now at the University of Arizona in Tucson who has collaborated with Villems.

According to what Villems calls the "Tartu school," the emerging picture differs from the mainstream view. Villems, along with his research group, particularly Toomas Kivisild, has been publishing research indicating that *Homo sapiens* migrated from Africa to India and "incubated" there about 60,000 years ago before spreading out to people the rest of the world. The theory "is completely their own," says Peter Forster, a molecular anthropologist at the University of Cambridge, U.K., and "it has been gaining a surprising amount of acceptance." Forster says it would force a major revision of the field if it bears out.

Championing science

Soon after the Soviet Union crumbled, Villems became a scientist-diplomat for his country, first by negotiating Estonia's early entry into the E.U.'s research funding scheme. Ergma believes that this "gave us a head start" over the other former Soviet states. Now Estonia boasts one of the best Internet networks in Europe as well as a small but fast-growing high-tech industry.

At the top of Villems's to-do list at the Estonian Academy of Sciences, which holds sway over the government's science policies, is "to secure our place in the European Research Area." His experiences in Moscow were excellent preparation. In a flashback to the days when Estonian scientists had to fight for a piece of the pie within the Soviet Union, their focus is now on Brussels, where the E.U.'s $22 billion scientific budget is divvied up among its 25 member states. Ergma agrees that winning this external funding is crucial. "Although Estonian salaries are low, so is the cost of living," she says. "But a centrifuge or a computer is just as expensive as elsewhere. So we desperately need structural grants."

A necessary step for Estonia to remain competitive, as Villems sees it, is to reduce what he calls the "mediocracy" in his country's science. Sounding like a draconian thesis adviser, he says that after years of Soviet exploitation, "some Estonian researchers have a sense of entitlement, that they should be funded without having to do excellent work." He plans to make sure that Estonian research institutions and projects are assessed by peer-review from outside the country.

Villems says another major problem to be tackled is Estonia's "missing generation" of scientists. The academy estimates that about 1000 students stampeded away from science into more lucrative fields such as business shortly after Estonia's independence. To achieve what Villems calls "critical mass" among the ranks, science education will be getting a boost to attract the first generation of Estonians who never knew communism. And an equally important strategy, says Villems, is to offer start-up grants to lure successful Estonian researchers back home after doing postdocs abroad.

Villems has his work cut out for him. Entering his office is like plunging into a cave made of paper: Books line every wall, and piles of articles cover every surface. Squeezing into the chair at his computer like a pilot climbing into a cockpit, Villems chuckles at the chaos around him: "I don't mind it."

—JOHN BOHANNON

John Bohannon is a writer based in Berlin.

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Academic Careers

Family Matters: Stopping Tenure Clock May Not Be Enough

University policies aimed at giving women time to have a family and a career are no match for the pressure to publish

As a rare woman faculty member at Stanford Medical School in the late 1970s, neurobiologist Carla Shatz put her quest for tenure ahead of her desire to start a family. But as she toiled away in the lab, working on a range of problems in developmental biology, her biological clock was ticking faster than she realized. By the time she earned tenure in her late 30s, her reproductive years had passed. "For 4 years, I tried every fertility treatment that was available," says Shatz, now 57 and a professor at Harvard University. "Nothing helped." The disappointment, she says, contributed to the breakup of her marriage.

In the past decade, dozens of universities have changed their tenure policies to accommodate the family needs of their faculty members. They've adopted rules that provide time off from tenure-track positions, created part-time tenure slots, and spread the gospel about the need to make room for family choices in the climb up the academic ladder. But those changes aren't making much of a dent in the cultural norms that put a premium on productivity, especially at the start of an academic career. Last month, at the annual meeting of the Association for the Study of Higher Education, two researchers who surveyed women faculty members around the country on their attitudes toward extended tenure summed up the problem in the title of their talk: Fear Factor.

Two for one. Dawn Lehman and Marc Eberhard, husband-and-wife civil engineers at the University of Washington, say that sharing one faculty slot has eased the pressure on childcare.

"Simply having a policy in the faculty handbook is not enough," says Lisa Wolfwendel of the University of Kansas in Lawrence, who presented the data with her colleague, Kelly Ward of Washington State University in Pullman. Although the fear that a tenure extension could hurt their

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career "could be unfounded," she says, "it is a fear nonetheless."

That fear, Wolfwendel says, is rooted in the idea that women who use such policies are somehow asking for special treatment. For many women of Schatz's generation, going off the clock wasn't a viable option. "Doing science and having children were considered mutually exclusive," says Shatz. And despite their growing presence in the sciences—women now earn 37% of U.S. Ph.D.s, up from 14% 30 years ago—many women who enter academia say they are still looking over their shoulders as they climb the career ladder.

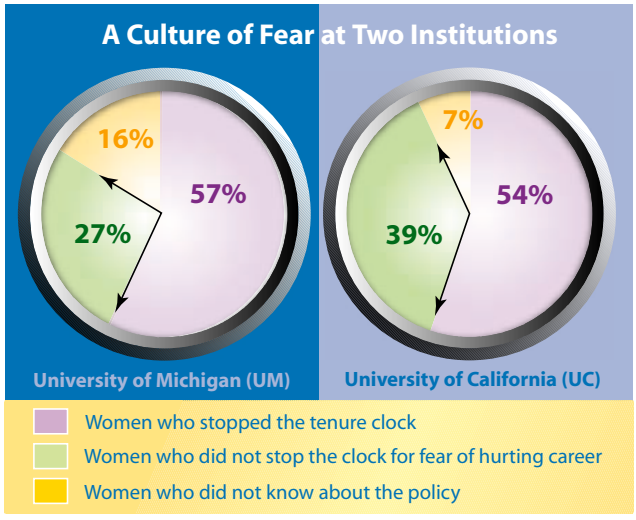
Two recent surveys at major research institutions point to the bind women faculty members face. Some 42% of women at the University of Michigan, Ann Arbor, for example, didn't request to go off the tenure clock even though they had reason to do so, and two-thirds of them said it was because of fear that an extension would have an adverse impact on their careers. "Had I stopped the tenure clock, I would have been viewed as weak by my senior colleagues," one faculty member wrote in her response, says study co-author Jean Waltman.

Women will go to great lengths to avoid that label, notes Waltman. Some reported that they had delayed pregnancy until after they got tenure. The survey also found that about one-third of the 86 women who had children did not request a lighter teaching load after giving birth.

A survey this fall at the nine University of California (UC) campuses found similar attitudes toward the school's tenure-extension policies. Although 48 women reported using it, 41 did not—most out of fear that it might derail their careers. Women who put their careers on hold, says one of the authors, UC Berkeley's Marc Goulden, must battle "the cultural conception that the faster you are, the better you are, particularly in the sciences. The expectation is that all the good people come up for tenure in 5 or 6 years, so God forbid if you take 7 or 8."

There are scant data on whether stopping the clock actually hurts a faculty member's chance of receiving tenure. Patricia Hyer, associate provost of Virginia Polytechnic Institute and State University in Blacksburg, says that none of the 10 women who have taken extensions for childbirth or other family-related reasons at her university since 1997 have been denied tenure. Lynn Singer, a psychologist and vice provost at Case Western Reserve University in Cleveland, Ohio, recalls having to admonish one tenure committee that had looked askance at the publication record of a candidate who had taken an extension. "I had to remind the committee that they needed to judge her productivity on the basis of her time on the clock," says Singer, adding that the woman was awarded tenure.

Even when the departmental climate is favorable, however, many women opt to defer pregnancy until after receiving tenure for fear of losing research momentum.



"Many scientists worry that grant reviewers will note the gap in productivity and go 'Oh, this person took a year's break, they aren't really serious,' " says a biologist at the University of Illinois, Chicago (UIC), who requested anonymity. Her own lighthearted attempt at addressing the issue, she says, has been "to insert my child's name and birth date in the chronological order of publications."

The UIC biologist also recommends that National Institutes of Health (NIH) grant applicants explain interruptions in their research. But biochemist Elvira Ehrenfeld, former director of NIH's Center for Scientific Review now back in the lab, is skeptical that such information would clarify matters—and it could even backfire. "Let's imagine an application to which one reviewer says, 'My enthusiasm was tempered when I saw that the researcher hasn't published anything in the past 2 years.' Then somebody else points out that the applicant started a family in those years," she says. "The scientific review administrator could then raise a very valid question: Do we give extra points to another applicant who had a baby but didn't stop publishing?"

Funding agencies could provide some critical help in child rearing, says Laurie Glimcher, an immunology professor at Harvard University. Glimcher, who remembers her struggle 2 decades ago to cope with child-care responsibilities as an NIH postdoc, recently lobbied successfully for a program at the National Institute of Allergy and Infectious Diseases (NIAID) to help scientists with early parenting. The $500,000 pilot program, announced in July, will enable principal investigators (PIs) to hire a technician for up to 2 years to assist a postdoc in their lab who has primary caregiving responsibilities. "We plan to make between eight and 10 awards," says Milton Hernandez, director of NIAID's Office of Special Populations and Research Training, who expects other NIH institutes to adopt the program.

And that's just a start, says Robert Drago, a labor economist at Pennsylvania State University, University Park, who studies bias against caregiving in the workplace: "From providing affordable housing near campus to subsidizing daycare, there's a lot that institutions should be doing if they mean business."

A few universities have reexamined how they do business, restructuring the tenure process to allow part-time tenure-track positions. The option has worked well for Dawn Lehman and Marc Eberhard, civil engineers who negotiated an arrangement 6 years ago with the University of Washington (UW), Seattle, to help the two balance career and family. A tenured faculty member, Eberhard offered to work half-time so that UW could create a half-time tenure-track slot for Lehman, who had just graduated from UC Berkeley. The department agreed. The arrangement enabled the couple to start a family and spend more than 30 hours of the workweek with their daughter, Collette, now 3. (They had a second child this fall.) Eberhard, who's Swiss-born, says the extra time off gives him a chance to teach his daughter French.

But splitting a job may not be enough for some young scientists. One 32-year-old postdoctoral fellow in physiology at a major research university in the Northwest has decided to pursue a non–tenure-track job in academia so that she and her husband can begin a family. "I've been quietly observing the senior women in my field since graduate school," she says. "I don't see balance; I don't see much of a family life." Instead, she says about her PI, a divorced mother, "I see her at work all the time. I don't think I want to make that kind of sacrifice."

Shatz says the community must figure out how to meet the needs of the next generation of scientists if academic research is to remain an attractive career. "People have very different career and personal paths, and we need to be more creative in offering options," she says. "We cannot continue a culture where women are reluctant to have children during their most fertile years."

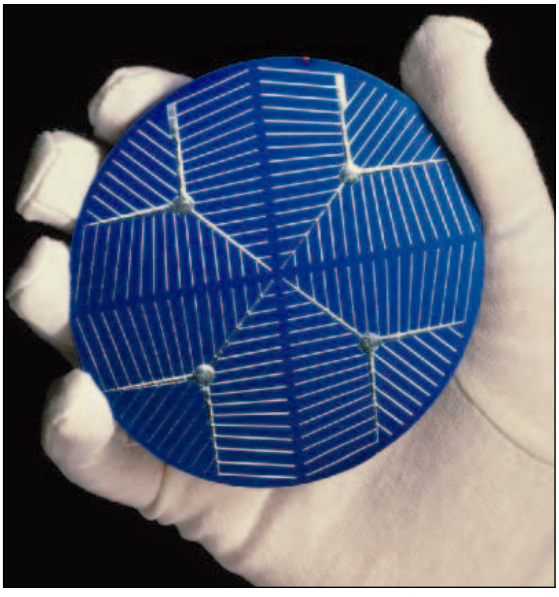
–YUDHIJIT BHATTACHARJEE

CREDIT: L. CREVELING/*SCIENCE*

Organic Solar Cells Playing Catch-Up

BOSTON, MASSACHUSETTS—Chemists, physicists, and materials scientists met here from 29 November through 3 December to talk about progress on everything from improved solar cells to engineering proteins.

Electronics researchers have made heady progress in recent years in turning organic materials into light emitters and computer logic circuitry. But their dream of matching these advances with novel organic solar cells that are cheap and easy to make has been stymied by their low rates in converting sunlight to electricity. At the meeting, however, Stephen Forrest of Princeton University and his student Jiangeng Xu shined a new ray of hope for the future of organic solar cells by boosting their efficiency from a few percent to 6%.

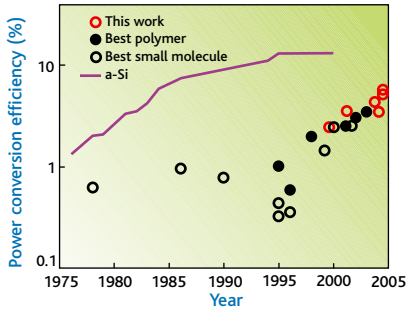


Still champion. Solar cells made from crystalline inorganic materials still dominate the marketplace.

"That's a very important development," says Ching Tang, a physical chemist at Eastman Kodak in Rochester, New York, whose team developed the first organic-based solar cell in 1986. Still, experts say that efficiency rates for organics will likely have to top 10% to have a shot at cracking the solar cell market.

Just getting to 6% was hard enough. Among the biggest problems was a Catch-22 involving the materials' poor absorption properties. To give the cells a better crack at grabbing incoming photons from the sun, researchers would like to lay photon-absorbing organics down in a thick layer. But if the layer is too thick, the absorbed photons generate heat before they can be converted into electricity. Once a photon is absorbed, it creates a particle known as an exciton—essentially just an excited electron bound to its opposite, a positively charged electron vacancy, or "hole." To generate electricity, these excitons must find their way to a material boundary that is energetically tuned to split them into their opposite charges, which then must find their way to oppositely charged electrodes. Unfortunately, excitons typically travel only short distances before their component electrons and holes recombine and give off their excess energy as heat.

Forrest's group applied a multipronged solution to this and other problems. They started with layers of different photon-absorbing materials—a combination of copper phthalocyanine and carbon-60, tuned to allow excitons to travel as far as possible before recombining. Second, to increase the odds of separating the excitons' charges, they created an interface that increased the surface area between the absorbing materials and other organic layers designed to whisk the electrons and holes toward opposite electrodes. Next, because the intensity of light in the cell is highest about 100 nanometers from the negatively charged electrode, the researchers tweaked the spacing between that electrode and the photon-absorbing layer to ensure that the light's peak intensity would land in the sweet spot of the photon-absorbing material. Finally, Forrest's team stacked solar cells atop one another so that even if the first cell didn't absorb all the incoming light, the other cells would finish the job.



On the rise. The efficiency of organic solar cells is approaching that of amorphous silicon.

The resulting structure, which also appears in the 6 December *Applied Physics Letters*, led to a dramatic boost in efficiency. That efficiency is still too low for organic solar cells to make it to market, Tang says, but Forrest has plans for boosting it further by adding antireflective coatings to prevent light from bouncing off the device, among other things. Organic solar cell makers must also prove that their devices can withstand prolonged exposure to searing rooftop temperatures and rain, snow, ice, and wind. Still, says Tang, to get efficiencies as high as 6%, "you have to get everything right."

Can Organics Take On Flash Memory?

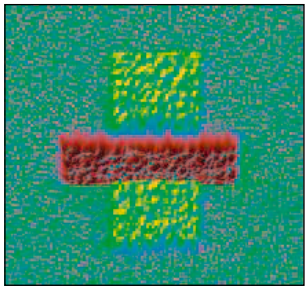
Improved solar cells aren't the only new devices on the horizon for organic materials. At the meeting, two separate California-based teams reported progress in turning organics into high-density, low-cost memory technology by using gold nanoparticles suspended in a simple polymer matrix. The new results are "a good first step," says Dimitris Tsoukalas, a physicist at the National Technical University of Athens in Greece, an expert on organic electronic materials. Polymer-nanocrystal memories, Tsoukalas says, have the potential to be cheap because they are much simpler to manufacture than silicon chips. They could also pack bits at a high density because organic memory cells should be easy to stack—unlike silicon devices. But Tsoukalas cautions that polymer-nanocrystal memories have a long way to go before they one-up today's silicon technologies, commonly known as flash memory.

In that technology, engineers grow specialized circuits, each of which contains two side-by-side silicon transistors. When electrons are sent through the first transistor (called the control gate), they push electrons onto the second (called the floating gate). That electron surge creates a digital "1" or "0" that can be preserved for years, read out, or rewritten by applying another electrical voltage. The reading and writing process, however, is slow, and the density of such memory cells is limited by the technology used to pattern them, which many experts see hitting a wall sometime after 2010.

So far organic materials haven't had much to offer as potential successor technologies.

CREDITS (TOP TO BOTTOM): ROSENFELD IMAGES LTD./PHOTO RESEARCHERS; ADAPTED FROM C. WRONSKI

At the meeting, however, the two groups—one led by University of California, Los Angeles (UCLA), materials scientist Yang Yang, and the other by Luisa Dominica Bozano of IBM's Almaden Research Center—reported that polymer-nanocrystal memory devices are easy to make, fast, and potentially high density. The devices consist of a simple sandwich of metal electrodes above and below a thin polymer film of polystyrene mixed with gold nanoparticles. The UCLA group—whose results also appear in the current issue of *Nature Materials*—also mixed in another electron-ferrying organic compound called 8-hydroxyquinoline (8HQ).



To a T. Applying different voltages alters conductivity of organic material (red and green).

When a "write" voltage of about 3 volts is applied to the device, electrons hop from the 8HQ molecules and onto the gold nanocrystals. Once the electrons arrive, they increase the conductivity of the polymer layer when a lower "read" voltage is applied because they make it easier for the electrons to hopscotch between the electrodes. The IBM group used much the same strategy but tested dozens of different combinations of the polymers, nanocrystals, and electrodes.

The next step for researchers, says Yang, is to demonstrate that polymer memories are long lived and robust. If they can manage that, the computer memory field may soon find itself with some new competition.

Protein Engineers Go for Gold

Proteins have become biochemists' favorite Christmas tree, perfect for decorating with all kinds of molecular ornaments. In recent years, teams around the globe have induced the cell's protein factories, called ribosomes, to append protein chains with novel amino acids and other organic groups. Now researchers from the Massachusetts Institute of Technology and the University of Texas (UT), Austin, have managed to get ribosomes to decorate proteins with inorganic gold nanoparticles as well. The development could open the door to new tests of the construction abilities of ribosomes, as well as novel ways to image proteins.

"It sounds pretty cool," says Steven Benner, a biochemist at the University of Florida, Gainesville, whose lab has pioneered "synthetic biology" techniques to expand the genetic alphabet in hopes of synthesizing novel proteins. Benner notes that gold particles tend to show up well with high-resolution imaging techniques, such as transmission electron microscopy. That could make the tiny gold tags useful for nailing down the structure of proteins, such as those imbedded in cell membranes, that are difficult to image with conventional protein-imaging techniques such as x-ray crystallography. And studding proteins with different-sized nanoparticles should give researchers a new way to investigate basic questions about the ribosome, such as how large a particle these factories can work with.

The new work builds on earlier experiments in which chemist Angela Belcher and colleagues at UT evolved bacterial proteins capable of binding different types of inorganic nanoparticles (*Science*, 24 December 1999, p. 2442). But the technique couldn't control exactly where on the proteins the nanoparticles ended up. At the meeting, Belcher's student Ioana Pavel reported that they had solved the problem, thanks to considerable help from ribosomes. These protein-building factories read the nucleic acid code of messenger RNA (mRNA), which itself is translated from DNA, and follow the instructions to assemble amino acids in their programmed sequence. In this process, known as translation, mRNA's instructions appear as a string of three-letter words called codons. In the ribosome, another set of RNA molecules called transfer RNAs (tRNAs)—which have mRNA-reading "anticodons" on one end and carry the corresponding amino acid on the other end—translate these codons into the specific amino acids that the ribosome then knits together.

Transfer RNAs, however, don't normally add inorganic nanoparticles to proteins. So Belcher, who is now at the Massachusetts Institute of Technology, in conjunction with biochemist Karen Browning's lab at UT, decided to make their own tRNAs that could do the job. They took advantage of the fact that the amino acid cysteine harbors a reactive sulfhydryl group, which has a strong affinity for gold. They started by using standard molecular biology techniques to generate large amounts of tRNA loaded with the cysteine. They then reacted the cysteine tRNA with the gold nanoparticles in a way that added a single gold particle to each cysteine. Next, they prepared an extract from *Escherichia coli* that contained ribosomes as well as all the other protein-building biomolecules needed except cysteine tRNAs. They then added their gold-tagged cysteine tRNAs to the soup. Finally, they added the mRNA that coded for a protein known as green fluorescent protein, which normally contains two cysteines in its protein chain. And as the ribosomes knit copies of the protein together, they added the gold-tagged cysteines, decorating each protein with two nanoparticles, a result that showed up clearly in subsequent imaging tests.

Down the road, Benner and others say that such inorganic tags could provide imaging signposts that would tell them exactly how far apart the nanoparticles wind up when they are incorporated into proteins—potentially critical information for working out the structures of highly complex proteins. If so, that would be a welcome gift to many structural biologists.

–ROBERT F. SERVICE

Snapshots From the Meeting

Crime Busters. Law enforcement agencies have long dreamed of using semiconductor terahertz lasers to hunt down weapons because the beams readily penetrate clothing and other materials. Progress has picked up in recent years, as researchers have made the first semiconductor terahertz lasers. Unfortunately, these operate at hard-to-achieve ultralow temperatures. At the meeting, researchers from Sandia National Lab reported a semiconductor "quantum cascade" terahertz laser that operates at 137 kelvin, the highest temperature yet, a key step on the road to room-temperature operation.

Crime Busters, the Sequel. Computer hard disks store and retrieve data by using tiny devices that convert magnetic fields into electrical signals. Now researchers at Stanford University are trying to use the same devices to detect molecules. The researchers attached magnetic beads to snippets of single-stranded DNA. They then showed that they could detect a change in the magnetic signal when these snippets bound to their DNA partner strands. Such devices could revolutionize police work by making it possible to instantly identify DNA at a crime scene.

Improving water purification. A mere 0.5% of the water on Earth is both fresh and accessible. So better membranes to filter out pathogens, toxins, salt, and other contaminants in water could help quench the planet's thirst. Researchers at MIT presented one new approach: two-part polymer films, with hydrophobic groups that lend stability and hydrophilic portions that create a regular array of tiny holes for filtration. The result is a membrane that allows more water to flow through.

CREDITS: Y. YANG/*NATURE MATERIALS* 3, 918-922 (2004)

Random Samples

Edited by Constance Holden

Deliver Us From Evil

Anxiety producer.

Reminding voters of their mortality may induce them to lean toward a charismatic leader, a new study suggests.

Sheldon Solomon, a psychologist at Skidmore College in Saratoga Springs, New York, and colleagues asked 95 college students to write answers to anxiety-producing questions such as "What [do] you think will happen to you as you physically die?" Another 95 were asked to write about their next important exam. Then all subjects were asked to vote on campaign statements by three politicians: one emphasized the nation's greatness and victory over evil, one focused on interpersonal cooperation, and one emphasized achievement of specific goals.

The task-oriented candidate got a plurality of votes in both groups. But 30% of the subjects who had been thinking about death voted for the charismatic leader, compared with only 4% of the controls, the authors report in the December issue of *Psychological Science*. Solomon says the results support an element of terror-management theory: that when confronted with the threat of death, people manage their fears by becoming more aggressive and choosing powerful leaders.

Daniel N. McIntosh, a social psychologist at the University of Denver, Colorado, says he was "struck by the magnitude of the effect," although he adds that the difference between the two groups might have been less if subjects had had other outlets to express their anxiety.

Robbing Peter to Pay Phil

Punxsutawney Phil, the groundhog that lets us know each February how long winter will last, came to Washington, D.C., last week to defend some pork: a congressional appropriation of $100,000 for a new weather science museum in his Pennsylvania hometown.

Peterson and pork defender.

Advocates for the museum claim it would be the first to explore "the science and folklore of weather prediction." Representative John Peterson (R–PA), author of the earmark, sees the center, scheduled to open in 2006, as a boon to the economically depressed region.

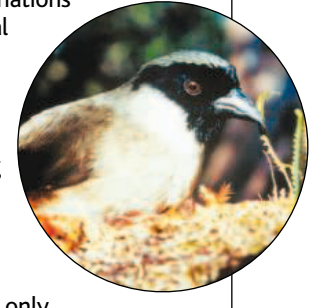
But opponents of such earmarks say projects like Phil's are taking money from more legitimate pursuits. The Punxsutawney Weather Discovery Center (weatherdiscovery.org) is funded from the same pot that funds NASA, the Environmental Protection Agency, and the National Science Foundation (NSF). It still needs another $500,000 to build and display exhibits. Jayme Organ, the museum's only full-time employee, says the National Science Foundation is a logical source—"but I've heard that they are cutting back because of a tight budget." NSF's 2005 budget was cut by 2%.

Race and Immunity

Here's another log to stoke the race-and-medicine debate. Variations in genes that regulate inflammatory responses may help explain why blacks and whites seem to have different susceptibilities to some disorders, according to researchers at the University of Pittsburgh, Pennsylvania.

The team, headed by epidemiologist Roberta B. Ness, took DNA samples from 179 African-American women and 396 white women who had undergone prenatal care and normal deliveries at a Pittsburgh hospital. The scientists evaluated the samples for variants of genes that encode inflammation-regulating proteins—called cytokines—that are secreted by the immune system. The African Americans were significantly more likely, by ratios of up to 5 to 1, to carry four genetic variants known to increase the inflammatory response, the researchers report in the 1 December issue of the *American Journal of Epidemiology*. They also had more of the gene variants that dampen the release of anti-inflammatory proteins—"kind of a double whammy," as Ness put it.

The researchers point out that inflammation is a common element in a host of conditions—heart disease, stroke, diabetes, and kidney disease, as well as premature labor, transplant rejection, and certain autoimmune disorders—that disproportionately affect blacks. Molecular biologist Joel Buxbaum of the Scripps Research Institute in La Jolla, California, who studies mutations associated with heart disease, says that the study is interesting but that it would be useful to know if the genetic variations correspond with actual cytokine levels.



Conservation Cliffhanger

Things are not looking good for the Po'ouli (right), one of the most endangered of Hawaii's native birds. On 26 November, the only individual in captivity died, apparently of old age. Only two more are thought to remain in the wild, and they haven't been seen since February.

One of the 15 species of endemic honeycreeper that still survive in Hawaii, the Po'ouli was discovered in 1973, when it had an estimated population of 200. The Maui Forest Bird Recovery Project has been trying to find the two known remaining birds in the rugged Hanawi Natural Area Reserve. But after a 9-day search, researchers returned last week empty-handed. They aren't even sure of the sex of these two birds, which are at least 7 years old—not a good age for captive breeding. Nonetheless, "we haven't given up hope entirely," says ornithologist Kirsty Swinnerton, who plans to return to the reserve next February.

CREDITS (TOP TO BOTTOM): DIGITAL VISION; P. BAKER; PABLO MARTÍNEZ MONSIVAIS/AP

Edited by David Grimm

AWARDS

Historical honor. Scott Gilbert has won the 2004 Alexander Kowalevsky Medal from the St. Petersburg Society of

Naturalists "for extraordinary achievements in comparative zoology and embryology." Gilbert, a developmental biologist at Swarthmore College in Pennsylvania, is author of the popular textbook *Developmental Biology* and currently studies the evolutionary origins of the turtle's shell.

The award was established in 1910 to honor the Russian embryologist and early Darwinian who first showed the close evolutionary relationship between vertebrates and tunicates, small sea creatures with a primitive backbone. The first medal wasn't awarded until 2001, however, and its monetary value—a professor's yearly salary in 1910—is now about $8.75. "It only makes the academic honor more valuable," Gilbert says.

Riding a wave. A childhood fascination with gyroscopes has paid off handsomely for Aaron Goldin, a senior at San Dieguito High School Academy in Encinitas, California. Goldin won first place—and a $100,000 scholarship—in the individual category of the Siemens Westinghouse Competition last week for inventing a wave-powered generator based on a gyroscope.

Exposed at an early age to tinkering by his father, Goldin set out to use a gyroscope to convert the rocking motion of waves into electricity and eventually produced a toaster-sized prototype that cranks out 0.8 watts—enough to power radio transmissions from a buoy or life raft. Goldin hopes the device could be scaled up to become a source of renewable energy.

"The ingenuity is quite remarkable," says Scott Miller, a judge and cell biologist at the University of Utah, Salt Lake City.

JOBS

Team effort. Geologist Arabinda Mitra has been named the first executive director of the Indo-U.S. Science and Technology Forum. The 4-year-old forum, funded by both governments and based in New Delhi, has sponsored workshops on genomics, brain research, and high-performance computing and next month will host 100 young American and Indian scientists for a 3-day meeting in Bangalore. "The forum is perfectly placed for a solid takeoff under Mitra's leadership," says Norman P. Neureiter, co-chair of the forum and director of the Center for Science, Technology, and Security Policy in Washington, D.C., at AAAS, which publishes *Science*.

Tennessee bound. Paul Gilman, who stepped down 2 weeks ago as head of science at the Environmental Protection Agency, has a new gig as the first director of the Oak Ridge Center for Advanced Studies.

The Tennessee center was conceived in 2000 when the University of Tennessee and Battelle won a bid to manage the Department of Energy's Oak Ridge National Laboratory. As part of the deal, the two hooked up with other regional universities to launch a think tank focusing on science and policy issues. Although plans are still in flux, Gilman says he envisions a resident scholar program with teams working on topics from nanomedicine to energy and transportation: "The hope is to bring researchers from various sciences together with policy analysts."

A formal rollout is planned for next month. "I'm sure [Gilman] will turn [the center] into something terrific," says

James Reisa, who directs the Board on Environmental Studies and Toxicology at the National Academies, where Gilman had served previously as director of life sciences and agriculture.

DEATHS

Math giant. Shiing-Shen Chern, who revolutionized the field of differential geometry and became one of the central figures of modern mathematics, died on 3 December at his home in Tianjin, China. He was 93.

Born in China and trained in Germany before coming to the United States, Chern developed a set of algebraic principles, later called Chern classes, that helped bring geometry out of the two-dimensional world and played a fundamental role in string theory. Chern co-founded the Mathematical Sciences Research Institute at the University of California, Berkeley, in 1982 and won the U.S. National Medal of Science as well as the Wolf Prize in mathematics.

"He was a towering figure in 20th century mathematics," says longtime colleague and Berkeley mathematician Calvin Moore, who also recalls his "uncommon kindness and generosity."

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Psychiatric Treatment for Great Apes?

HUNDREDS OF GREAT APES, MAINLY CHIMpanzees, are in laboratories, zoos, and private homes. A growing movement suggests that chimpanzees who are no longer needed for research ought to be retired to sanctuaries with limited access to the public. Many of these chimpanzees show behavioral abnormalities (due to sensory, motor, or nutritional deprivation; social isolation; experimentally induced lesions; or aging) that pose difficulties for integrating them into existing or new social groups (*1*, *2*).

Here we propose that (i) captive great apes suffer from disorders homologous to human psychopathology; (ii) such conditions are in principle treatable; and (iii) our closest relatives in the animal kingdom deserve proper psychiatric treatment.

Flower, a former research chimpanzee, is in a temporary sanctuary and will move into a permanent sanctuary in a few months.

CREDITS: MARGARET WILSON/CHIMP HAVEN, INC.

In experimental conditions, young primates who are separated from their mothers shortly after birth show pathological anxiety or depression-like states (*3*). As adults, these individuals are often neglectful mothers (*4*). Also, captive primates in conditions of sensory deprivation or social isolation often develop cage stereotypies (captivity-induced excessive repetitive movements such as body-rocking, hair-pulling, or pacing); self-mutilation; pica syndrome (perverted appetite syndrome characterized by compulsive ingestion of inedible organic or nonorganic matter); and other behavioral abnormalities (*5*).

In contrast, such symptoms of psychopathology have not been observed in decades of field studies of wild populations. Case reports of orphaned juvenile chimpanzees suggest, however, that they may develop severe depression-like states despite being old enough to forage for themselves (*6*).

In light of the close genetic relationship of the great apes and humans, we suggest that phenotypically similar, possibly homologous disorders are associated with comparable dysfunctions at the cellular and neurotransmitter level in the brain and hence may respond similarly to treatment (*7*).

Because environmental enrichment alone may not suffice to treat behavioral abnormalities in apes (*8*), additional psychopharmacological treatment such as selective serotonin reuptake inhibitors for depression, pathological anxiety, and stereotypic movement disorder or acetylcholinesterase inhibitors in cases with cognitive decline may be effective in improving their mental health (*9*, *10*).

Despite possible ethical dilemmas, systematic psychiatric assessment and treatment of captive great apes can probably be done noninvasively, relying heavily on longitudinal ethological study. It is a logical extension of the debate about humane and ethical treatment of our closest relatives that we owe them proper psychiatric treatment to reduce their anthropogenic suffering.

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Stopping the Spread of Drug-Resistant Malaria

C. ROPER *ET AL.* REPORT THE DISTURBING dissemination of pyrimethamine-resistant *Plasmodia* (the parasite that causes malaria) from Asia to Africa ("Intercontinental spread of pyrimethamine-resistant malaria," Brevia, 20 Aug., p. 1124). They recommend that travelers moving between Southeast Asia and Africa be screened and treated as a possible means for preventing this dissemination. Security screening at points of entry and exit is already cumbersome, and additional infectious disease screens, requiring invasive sampling and laboratory testing, are unlikely to be acceptable. The large number of travelers who do not move between Africa and these endemic areas by direct routes or even connecting itineraries will be missed. A voluntary screen is also unlikely to be useful because when travelers from endemic areas feel ill, they commonly do not seek medical care from official sources (*1*). Asymptomatic carriers would be even harder to police, and a system of attempting to control resistant malarial parasites at borders also opens the question of the necessity for prescreening and treating travelers and vectors of other drug-resistant pathogens.

The second challenging strategy suggested by Roper *et al.*—minimizing the foci of resistant pathogens in both Asia and Africa—may be more practicable, is less likely to infringe on human rights, and would provide benefits for endemic as well as threatened countries. Although strategies for the containment of resistant organisms have been proposed (*2*), prioritization and implementation of these strategies are far from optimal. Thus, modus operandi for dealing with impending biological threats, such as those posed by future artemisin-resistant *Plasmodia*, for example, do not exist. A necessary first, but not only, step would be improving routine surveillance of existing resistant organisms, as well as those yet to emerge, particularly in developing countries.

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Response

WE WELCOME OKEKE'S LETTER AND AGREE with most of its content. There are three possible approaches to preventing intercontinental spread of resistant parasites. First, we can minimize the foci from which spread can occur; second, we can limit the establishment of resistant migrant parasites; and third, we can prevent resistant pathogens from traveling between continents. Given that imported antimalarial drug resistance to both chloroquine and pyrimethamine has resulted in a dramatic increase in malaria mortality in Africa (*1*, *2*) and that future importation of new drug resistance mutations has the potential to accelerate malaria death rates, we do not think that any one of these options should be discounted.

We already have an excellent tool for implementing the first two lines of defense. Artemisinin combination therapies (ACT) (*3*) are extremely potent antimalarials that can minimize both the spread of resistance and establishment of resistant migrant parasites. We agree with Okeke and the World Health Organization that ACT should be introduced as widely and rapidly as possible in endemic areas, and we hope that our Brevia describing intercontinental spread of pyrimethamine resistance will add extra urgency to this effort. But there is a downside to reliance on a single approach—what will happen when (not if) resistance to artemisinin compounds emerges?

What of the third option—preventing intercontinental travel of resistant parasites? We broadly agree with Okeke's assessment that passenger screening to detect and prevent pathogens from traveling would be challenging. However, we disagree that it would be ineffective, expensive, or unduly intrusive. There is a precedent in the case of yellow fever, where a certificate of vaccination—essentially an "infection-free certification"—is a legal requirement for entry into India, which is disease-free but where the mosquito vector exists. A malaria vaccine is not currently available; in the meantime, we can rely on detection of malaria carriers. Technical considerations are not a serious impediment to screening because malaria tests are cheap (<$1 per test) and rapid (<10 minutes) and could be completed before travel. Thermo-scanning (*4*) at airports was used to detect active SARS infections and could also be effective for malaria detection because most travelers from Southeast Asia or South America are malaria naive and would be symptomatic at low parasitemia.

We believe that a number of possible screening approaches could and should be considered. Imported resistance to malaria parasites has the potential to cause much greater mortality than the terrorist threats that are currently the focus of attention. However, because malaria deaths resulting from imported resistance will be less immediate and obvious, we concede that there is likely to be less political will to address this preventable problem.

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The Bush Administration and Climate Change

U.S. SECRETARY OF ENERGY SPENCER Abraham's Policy Forum "The Bush administration's approach to climate change" (30 July, p. 616) is founded on an error of omission and an error of economic understanding. The error of omission is to imply that President Bush's policy to reduce greenhouse gas (GHG) intensity will meaningfully slow GHG emissions. GHG intensity naturally declines in the increasingly service-oriented U.S. economy. For example, even as actual GHG emissions increased by 22.5% from 1990 to 2000, GHG intensity decreased by 34.7% (*1*). The nonpartisan General Accounting Office (GAO) projects that between 2001 and 2012, GHG intensity will decline 14% even with no emission reduction policy in place. Bush's voluntary approach, if successful, will lead to an additional reduction in GHG intensity in 2012 of only 4% and an actual increase in GHG emissions of over 13%.

Abraham also appears to misunderstand basic economics. Economic improvement would provide additional resources for society to deal with climate change, as he suggests. But Bush's approach, which allows us to emit GHG to the atmosphere for free, constitutes a subsidy for polluting activities and a drag on the economy. For example, warmer temperatures, sea level rise, more intense storms, and more severe droughts, which are all expected to accompany global warming, would cause economic damage to human society. Because we do not pay for that damage, we all see an incentive to pollute more than the economic optimum (where the economic optimum is the point at which the considerable benefits we reap from activities that result in GHG emissions equals the growing cost of those emissions). In such cases, even "costly" emission reduction schemes may constitute clear economic improvements.

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Reference
1. General Accounting Office (GAO), GAO-04-131T, 2003.

IN THE POLICY FORUM "THE BUSH administration's approach to climate change" (30 July, p. 616), U.S. Secretary of Energy Spencer Abraham states that the Administration's near-term goal is to "reduce the greenhouse gas intensity of the U.S. economy by 18% by 2012," that is, to reduce the amount of greenhouse gases emitted per unit of real gross domestic product by 18% by 2012. Although this is a substantial reduction in relative terms, it is essentially in line with how the U.S. economy has been performing in the recent past. According to the Pew Center on Global Climate Change (*1*), greenhouse gas intensity decreased by 21% during the 1980s and by 16% during the 1990s. An 18% goal for the coming decade is just slightly better than business as usual and would still cause a net increase in emissions of about 10% because of continued economic growth.

Although goals need to be realistic, it is equally important that they be ambitious. If a company like Dupont can reduce its greenhouse gas emissions by 65% since 1990 or an Alcoa believes it can cut its actual emissions by 25% by 2010 (*2*), surely, we can do much better than business as usual.

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References
1. See www.pewclimate.org/policy_center/analyses/response_bushpolicy.cfm.
2. "Global warming: Why business is taking it so seriously," *BusinessWeek*, 16 Aug. 2004.

Response

THE BUSH ADMINISTRATION BELIEVES THAT ITS goal of reducing the emissions intensity of the U.S. economy—measured as the ratio of total greenhouse gas emissions per unit of economic output—by 18% from 2002 to 2012 is ambitious but achievable. Although the nation's greenhouse gas emissions intensity improved 17.5% between 1990 and 2000 (*1*), the Energy Information Administration, taking into account current and anticipated factors in energy markets, projects an emissions intensity improvement of 13.8% from 2002 to 2012 (*2*). Achieving the president's goal, therefore, would increase the projected rate of improvement over the period by about 30%.

Higgins states that once the economic benefits and costs of greenhouse gas emissions achieve parity, costly emission reductions may make economic sense. Our present state of knowledge on climate change is insufficient to determine where that point is or when it might be reached. Reducing such uncertainty is a major feature of the Bush administration's policy. The Climate Change Science Program is addressing fundamental questions about climate variability and sensitivity to help clarify risks and benefits. The Climate Change Technology Program is working on advanced technologies to improve the performance and reduce the cost of mitigation options. We believe that this approach, coupled with near-term efforts to reduce emissions intensity, is sound and offers the greatest potential for addressing what is a long-term challenge.

SPENCER ABRAHAM

U.S. Secretary of Energy, 1000 Independence Avenue, S.W., Washington, DC 20585, USA.

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1. Energy Information Administration, Emissions of greenhouse gases in the United States 2001: Historical and projected U.S. carbon and total greenhouse gas intensity; *Emissions of Greenhouse Gases in the United States 2001* [DOE/EIA-0573 (2001)]; available at www.eia.doe.gov/oiaf/1605/gg02rpt/gas.html.
2. Energy Information Administration, Issues in focus: U.S. greenhouse gas intensity, *Annual Energy Outlook 2004* [DOE/EIA-0383 (2004)]; available at www.eia.doe.gov/oiaf/aeo/issues_8.html.

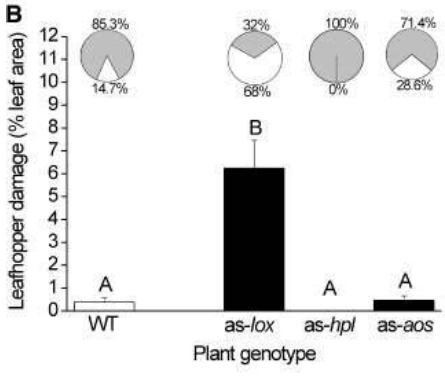
CORRECTIONS AND CLARIFICATIONS

News of the Week: "Advice on science advising leaves plenty of questions" by J. Mervis (26 Nov., p.1450). The article incorrectly reported the number of signers on a petition circulated by the Union of Concerned Scientists earlier this year that criticized the science policies of the Bush Administration. The number is 6000, not 60,000.

News of the Week: "...And NCI hears a pitch for biomarker studies" by J. Kaiser (12 Nov., p. 1119). The article incorrectly stated that a cancer biomarker initiative proposed by Lee Hartwell did not include a price tag. Hartwell estimated a cost of $20 million for the first cancer site, then $6 million for each additional site.

News of the Week: "Skeptics question whether Flores hominid is a new species" by M. Balter (12 Nov., p. 1116). Gadjah Mada University is in the city of Yogyakarta, Indonesia, not Jakarta, as stated.

Reports: "Silencing the jasmonate cascade: Induced plant defenses and insect populations" by A. Kessler *et al.* (30 July, p. 665). The shading of the second pie chart in Fig. 2B was reversed. The area representing the proportion of damaged plants (68%) should be displayed in white, whereas the proportion of undamaged plants (32%) should be displayed in gray, as described in the figure legend. The corrected figure is shown here.



TECHNICAL COMMENT ABSTRACTS

Comment on "Uracil DNA Glycosylase Activity Is Dispensable for Immunoglobulin Class Switch"

James T. Stivers

Begum *et al*. (Reports, 20 August 2004, p. 1160) concluded that uracil DNA glycosylase (UNG) activity is not required for class switch recombination (CSR) based on experiments with UNG mutants. However, the residual uracil removal activities of UNG single mutants may be sufficient to introduce double-strand breaks and induce CSR.

Full text at www.sciencemag.org/cgi/content/full/306/5704/2042b

Response to Comment on "Uracil DNA Glycosylase Activity Is Dispensable for Immunoglobulin Class Switch"

N. A. Begum and T. Honjo

Negligible levels of uracil (U) removal activity in mouse UNG single mutants cannot explain CSR rescue activity because CSR rescue activities of wild-type UNG and single mutants are equivalent per given amount of protein. We argue that UNG is not involved in U removal and that UNG double mutations reduce the affinity of UNG to DNA or other repair enzymes critical for CSR without changing the gross structure.

Full text at www.sciencemag.org/cgi/content/full/306/5704/2042c

HISTORY OF SCIENCE

Bragg Reflections

John Meurig Thomas

In 1966, when the Royal Society bestowed the Copley Medal, its highest honor, on William Lawrence Bragg, its president, Patrick Blackett, remarked: "The striking characteristic of Bragg as a scientist has been his direct and simple approach to complicated physical situations; his solutions of problems have a lucidity and simplicity which, in retrospect, make one forget how baffling they often seemed in advance." How apt, for belief in the essential simplicity of things was one of W. L. Bragg's scientific articles of faith. His former students still vividly recall his pellucid lecture materials and his devastatingly effective demonstrations, especially in optics. To lay audiences and to children, whom he addressed with avuncular charm, he was a superb and riveting expositor because of his exceptional skill in reducing scientific advances to their simple, quintessential nature.

Light Is a Messenger
The Life and Science of William Lawrence Bragg
by Graeme K. Hunter
Oxford University Press, Oxford, 2004. 323 pp. $59.50, £35. ISBN 0-19-852921-X.

Remarkable as these talents were, it was Bragg's brilliant insight as a 22-year-old graduate student that secured his immortality in the annals of physics. That insight and a subsequent sequence of ingenious experiments led to his receiving, at age 25, the 1915 Nobel Prize in physics (which he shared with his father, William Henry Bragg). He remains the youngest ever Nobel laureate. Graeme Hunter, a professor at the University of Western Ontario, catches many of W. L. Bragg's charms and graces in the first biography of the man, *Light Is a Messenger*.

Walking along the Backs in Cambridge in the autumn of 1912, Bragg had an idea that led immediately to a dramatic advance in physics and has since transformed chemistry, mineralogy, materials science, biochemistry, and molecular biology. He realized that the phenomenon of x-ray diffraction, reported by Max von Laue earlier that year, can be interpreted very simply as arising from reflections of the x-rays by planes of atoms within the solid and hence that the consequential diffraction patterns provide quantitative information on the arrangement of atoms in a crystal. His interpretation led to the formulation of Bragg's law—$n\lambda = 2d \sin\theta$—an equation (*1*) as widely used as any in present-day practical physics and crystallography.

The Braggs' early papers on the structure of some minerals as indicated by their diffraction patterns were greeted with shock and exhilaration. Shock, because the Braggs inexpugnably established that there is no molecule of sodium chloride in rock salt, simply an extended alternation of sodium and chloride ions. Exhilaration, because the structure of diamond confirmed the tetrahedral coordination of carbon envisaged by Jacobus van't Hoff and others 40 years earlier.

W. L. Bragg was born (1890) in Adelaide, Australia, where his father was professor of physics. He entered the university there at age 15, graduated in mathematics 3 years later, and became a student at Cambridge in 1909. Following his sensational achievements there, he served with distinction in World War I before returning to academia. He was only 29 when he succeeded Ernest Rutherford at the University of Manchester, where he made numerous crucial discoveries concerning the structure of minerals (especially silicates), metals, and alloys and, in association with Evan Williams (whom Bragg regarded as "a volatile genius"), order-disorder phenomena. He also pioneered new procedures of x-ray analysis, notably demonstrating how electron densities in crystalline solids (and therefore in ions and molecules) may be retrieved from diffraction data.

He again succeeded Rutherford when, in 1938, he returned to Cambridge as the Cavendish professor, a post for which he had yearned over many years. His appointment disappointed many, especially the particle physicists, and he himself was conscious of inherent differences in personality and management style between him and the autocratic Rutherford. Nonetheless, in retrospect he is seen to have fostered areas of physics that have profoundly influenced the growth of the subject and its impact on numerous other fields. He greatly encouraged the work of Max Perutz on hemoglobin and John Kendrew on myoglobin. When these two chemists solved the structures of their fiendishly complicated molecules using the heavy-atom technique to tackle the phases of their diffraction peaks, Bragg, ever the master of metaphor, said that the protein molecules take no more notice of a heavy atom than "a maharajah's elephant would of the gold star painted on its forehead." Radio astronomy is another major field of physics initiated (by Jack Ratcliffe and Martin Ryle) at the Cavendish laboratory in Bragg's day and much supported by him. Both lines of research led to Nobel awards: Perutz and Kendrew shared the 1962 prize in chemistry; Ryle and Antony Hewish, the 1974 prize in physics.

In the 1940s, Bragg's intellectual perspicacity and elegant simplicity of method enabled him and a graduate student, John Nye, to demonstrate the ease of movement and alignment of edge dislocations in metals and alloys through the agency of the "bubble raft" technique (which uses soap bubbles of uniform diameter on the surface of a liquid).

50 years on. The 1965 Nobel laureates included (left to right, back row first) Richard Feynman, Robert Woodward, Jacques Monod, André Lwoff, François Jacob, Julian Schwinger, and Mikhail Sholokhov. Bragg (front right) gave a commemorative lecture at the ceremonies.

Independently of William Shockley, he discovered how dislocations in simulated metals dissociate into partials and generate stacking faults. In the 1950s, he was overall head of the laboratory that accommodated Francis Crick and James Watson, who were members of the Perutz-Kendrew team that the Medical Research Council (through Bragg's catalytic encouragement) had established within the Cavendish laboratory. He resigned from his post in 1953 to become director of the Royal Institution of Great Britain and the Davy Faraday Research Laboratory there—posts to which W. H. Bragg had been appointed 30 years earlier.

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At the Royal Institution, he encouraged David Phillips's landmark work in solving the structure of lysozyme (the first enzyme to be thus conquered), and he saw how Phillips and Louise Johnson could explain the mode of operation of this enzyme.

Hunter's book has many praiseworthy features: it is well written and draws from an enormous body of fact, from the personal to the professional. The background from which Bragg emerged, his often strained yet basically happy relationship with his father, and his rivalry with Linus Pauling are all sensitively expressed. The fury of Pauling, Crick, and Maurice Wilkins when they were shown the first draft of Watson's *The Double Helix* (to which Bragg contributed the foreword) as well as the turbulence that led to the departure of Bragg's immediate predecessor at the Royal Institution (Edward Andrade) make fascinating reading. One also learns that Bragg, surprisingly, was susceptible to stygian moods of depression.

Although the general trajectory of Bragg's life is commendably described, Hunter's treatment of the science and his judgments concerning Bragg's place in history are less satisfactory. The diagrams he uses are frequently misleading. For example, figure 0.1 talks of "spectra" where "diffraction" should be used, and figure 0.2 (on the relation between atomic planes and the position of corresponding spots on x-ray diffraction patterns) is misleading to the point of opacity. Throughout the book Hunter calls the alkali metal halides the "alkaline halides," an expression never used by chemists. He sensibly invokes the pedagogic statements of the late Charles Taylor in his Introduction, but how much better it would have been if he had also used some of the beautiful optical diffractograms that Taylor and others used to convey the nuances of crystallographic argument to the uninitiated reader. He could also have used some of the analogies that Bragg himself had included in his elegant article—redolent of his insights and charm—"British Achievements in X-ray Crystallography" (*2*).

Hunter's most disputatious claim is that Bragg's "work had no great influence on the physics of his time—or after. Considered against the great achievements of the first half of the twentieth century—quantum theory, relativity, and the structure of the atom—Bragg's contribution to the physics of the solid state seem relatively minor." To buttress his claim, Hunter tendentiously quotes both William Cochran and Crick. He also writes "before his work on minerals, it was understood that the silicates were complex crystals of silicon, metals, and oxygen." With criticism of this kind, one could equally argue that well before Crick and Watson's work on DNA, it was understood to be a complex molecule made of carbon, hydrogen, oxygen, nitrogen, and phosphorus.

It is true that Bragg, unlike the polymathic Linus Pauling, never mastered quantum mechanics and the structure of the atom. But do quantum mechanics and the intricacies of the atomic nuclei shed more light on the structure of living matter or the processes that synthesize proteins than do the succession of techniques initiated by Bragg?

Hunter's account does not capture the qualities of indefatigable enthusiasm, encouragement, and generosity of spirit that Bragg possessed to a singular degree. *The Double Helix* portrays Bragg as something of a fuddy-duddy; in his foreword, Bragg notes, inter alia: "Those who figure in the book must read it in a very forgiving spirit." Although Hunter describes this magnanimous gesture, he does not include the remarkable story told by Perutz of Rutherford's hostility toward J. D. Bernal (Perutz's Ph.D. supervisor). The conservative and puritanical Rutherford detested the undisciplined Bernal, a communist and a woman chaser who let his scientific imagination run wild. Rutherford wanted to

BROWSINGS

'Twas the Night

'Twas the night before Any Thing, and all through deep space,
Nothing existed—time, matter, or place.
No stockings, no chimneys. It was hotter than hot.
Everything was compressed in one very dense dot.

When out of the nothing there appeared with a clatter
A fat guy with reindeer and something the matter.
His nose was all runny. He gave a sick hack.
"Oh, Dasher! Oh, Dancer! I can't hold it back!"

He huffled and snuffled and sneezed one AH-CHOO!
Then like ten jillion volcanoes, the universe blew.
That dense dot exploded, spewing out stars,
Earth, Venus, Jupiter, Uranus, and Mars,

Helium, hydrogen, the mountains and seas,
The chicken, the egg, the birds and the bees,
Yesterday's newspaper, tomorrow's burnt toast,
Protons and neutrons, your grandma's pork roast.

The universe expanded. The guy said with a wheeze,
"Who will ever believe the world started by sneeze?
So let's call it something much grander, all right?
Merry BIG BANG to all! And to all—Gesundheit!"

Science Verse. *Jon Scieszka, illustrated by Lane Smith.* Viking, New York, 2004. 40 pp. + CD. $16.99, C$25.50, £12.99. ISBN 0-670-91057-0.

Modeling his text after famous poems, songs, and rhymes, Scieszka offers young readers (ages 6 and up) a slightly subversive introduction to science. He touches on such topics as evolution, the water cycle, parasites, food chains, the particle-wave nature of light, and black holes. The accompanying images combine painting and collage, and on the CD the collaborators read the verses aloud.

CREDIT: DETAIL OF ILLUSTRATION BY LANE SMITH FROM *SCIENCE VERSE*

throw Bernal out of the Cavendish laboratory but was restrained from doing so by Bragg. Had Bragg not intervened, Bernal would not have started his pioneering work with Dorothy Hodgkin in molecular biology, Kendrew and Perutz would not have solved the structure of proteins, and Watson and Crick would never have met. Science owes Bragg an incalculable debt.

References and Notes

1. The equation gives the relation among the x-ray wavelength (λ), the angle of incidence (θ), and the spacing between atomic planes in the crystal (d); n is an integer, the order of reflection.
2. W. L. Bragg, *Science* **131**, 1870 (1960).

DOI: 10.1126/science.1106446

BIOMECHANICS

Optimized for Chewing

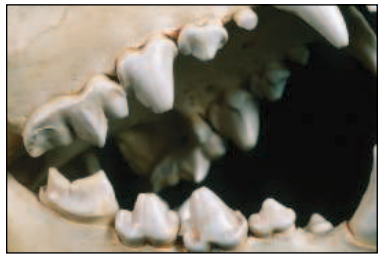
Natalia Rybczynski

The crowns of mammalian teeth exhibit an incredible diversity of shapes. Variable morphological features of teeth such as cusps, depressions, ridges, and infoldings are intuitively recognizable as having functional consequences, and they have been the subject of a considerable body of research. For most of the last 50 years, tooth function has been described in terms of "shearing," "grinding," or "crushing"—words referring to tooth geometry and the movement of the lower teeth relative to the upper teeth. However, Peter Lucas holds that the terms are of no relevance in dental functional morphology because they do not make any reference to food. "Without foods, all that can be done is to eulogize teeth for being so good at what they do." Lucas, an anatomist at the University of Hong Kong, sees this foodless perspective as "the central reason why studies of dental-dietary adaptation have remained stagnant."

The principal thesis of Lucas's *Dental Functional Morphology: How Teeth Work* is that the diversity of dental shapes among mammals is a consequence of variation in the mechanical properties of specialized diets. He considers fracture properties to be the most important because the chief function of most mammalian teeth is to break apart food particles to facilitate their digestion. Satisfying the high metabolic demands of mammals requires high rates of fracture and fragmentation, and as a result the correlation between mammalian dental morphology and diet is especially strong. Mammals are therefore an exceptional study system for investigating the form and function of teeth. Even so, the principles Lucas outlines in the book could also be applied to other toothy lineages.

Dental Functional Morphology
How Teeth Work
by Peter W. Lucas
Cambridge University Press, Cambridge, 2004. 371 pp. $130, £75. ISBN 0-521-56236-8.

In general, the book is written from an optimization perspective. Although the author often refers to evolution, readers interested in the roles of developmental or phylogenetic factors in the evolution of dental functional morphology will be disappointed. After an introductory overview, Lucas reviews oral and masticatory anatomy. Those familiar with these topics may wish to start with the fourth chapter, where the author presents the mechanical basics. He explains, for example, that the easiest way to propagate cracks through a particle is to use tensile forces to open cracks. He then places these mechanical principles in the context of real jaws. Jaws exert compressive forces, and compressive forces are generally the least effective way to produce fragmentation because they tend to close cracks or flaws rather than open them. To create tensile forces in food particles, jaws control the pattern of contact between the tooth and the particle so as to produce tensile forces, either through bending (e.g., across blunt cusps) or by driving blades or wedges (e.g., sharp cusps) through the particle. The optimal tooth shape for fragmenting particles depends on the properties of the food being processed (especially its toughness and elasticity), and Lucas offers specific hypotheses on these relationships. He includes an appendix that tabulates mechanical properties of teeth and potential foods, many of which have been previously unpublished.



Bone crushers. Spotted hyenas (*Crocuta crocuta*) use their blunt conical premolars to fracture bones.

Lucas also offers fresh insights into the importance of tooth size. Body size has long been recognized as having a profound effect on the form and function of organisms. For example, compared with small animals, larger animals generally have stouter lower limb bones and reduced metabolic rates per unit body mass (*1*). It has been recognized for some time that scaling patterns of mammalian dentitions cannot be explained in terms of standard body-size considerations, such as metabolic requirements. Molar sizes observed in large mammals, for instance, are much smaller than those predicted by body size (*2*). What accounts for the scaling patterns of tooth sizes? Lucas points to fracture mechanics as the likely missing link. Lucas argues that fracture failure is size dependent: larger particles fail at smaller stresses than smaller particles do. Consequently, tooth size should be more closely linked to the size of ingested food particles than to more standard variables such as metabolic body size. Not only does Lucas's "fracture scaling" offer new hypotheses for explaining variation in tooth morphology, it applies to other components of the chewing apparatus, such as jaw muscles, as well.

The book's strength lies in its focus on the application of engineering principles to questions of dental functional morphology. To this end, Lucas reviews many mechanical concepts within the main chapters. He also includes an appendix on "mechanical properties and their measurement." Subtitled "material properties made easy," this presents a reasonable overview of a difficult subject, although some discussions need to be tightened up and others clarified. For example, readers would have been aided by a more detailed explanation of the mechanism that underlies the relation between fracture and deformational domains. Very helpful aspects of the appendix are the descriptions of a mechanical testing machine ("the 'Darvell' HKU tester") and various approaches to determining the mechanical properties of mammalian foods in the field. The role of mechanical testing in studies of dental functional morphology is still in early development, and the appropriateness of the material tests Lucas proposes for understanding dental function remains to be verified experimentally. Nevertheless, the appendix offers a good point of departure for those interested in conducting such research.

Dental Functional Morphology presents an original and comprehensive overview of its field, and it provides a rich source of fresh ideas, including testable hypotheses. The mechanical engineering approach outlined in the book will certainly lead to new studies and insights into the functional significance of the diverse morphologies of vertebrate teeth.

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2. R. F. Kay, in *Development, Function and Evolution of Teeth*, P. M. Butler, K. A. Joysey, Eds. (Academic Press, New York, 1978), pp. 309–340.

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EDUCATION

Risks and Rewards of an Interdisciplinary Research Path

Diana Rhoten[1] and Andrew Parker[2]

Interdisciplinarity has become synonymous with all things progressive about research and education, not because of some simple philosophic belief in heterogeneity but because of the scientific complexity of problems currently under study (*1*). In many fields, it is argued, the easy work is finished as scholars are confronted with questions that defy easy categorization in or solution by traditional disciplinary frameworks. In response, myriad interdisciplinary programs have arisen, from federal-level initiatives such as the National Institutes of Health Roadmap and the National Science Foundation Integrated Graduate Education and Training program to campus-based endeavors like the University of Illinois Beckman Institute and the Stanford University Bio-X Program.

The rise of interdisciplinarity has also spawned a vast literature on how interdisciplinary research and training should be organized, how scientists and students will behave, and how activities of such programs could be facilitated (*2*–*6*). There have been, however, fewer studies that seek to understand empirically the links between institutional initiatives, individual attributes, and professional implications (*7*, *8*).

Between January 2002 and June 2003, we conducted surveys and interviews to analyze the interdisciplinary activities of researchers in five university-based programs funded under the NSF Environmental Research and Education portfolio (*9*). Entry into these programs was by application, invitation, and/or appointment.

We expected that because younger scientists are likely to have had more interdisciplinary exposure and less intellectual commitment to a particular field, they would be more predisposed toward these programs than their senior colleagues. At the same time, because senior faculty have accumulated greater professional freedom and more social resources, we thought that they would be more likely than their junior counterparts not only to affiliate with but also to collaborate in these programs.

Graduate students and full professors were indeed overrepresented in these programs as compared with other tenure-track researchers (see the table below) (*10*). However, apart from principal investigators who dominated large shares of interdisciplinary activity, graduate students demonstrated higher rates of interdisciplinarity than professors. Whereas 61 of 99 (62%) graduate students reported at least one interdisciplinary collaboration, only 72 of 147 professors (49%) claimed the same (*11*, *12*).

But, graduate students were also most likely to associate professional costs with interdisciplinarity. About 16% reported "negative" career effects of the program's "interdisciplinary" design (see the table). In describing real or perceived effects, graduate students indicated long-term costs. One described his position as "non-traditional, highly beneficial, but completely risky in the long run." Another explained: "For those of us who begin interdisciplinary, we get to design a [personal] renaissance to meet the needs of real-world problems. This renaissance, however, comes at a price—it may take us longer to establish ourselves in our careers." Several pointed to the greater prevalence of interdisciplinary role models among staff without tenure versus those with tenure.

When asked why they were willing to take these professional risks, graduate students frequently mentioned societal benefits. One student said "I have become very aware of the horrible inefficiency of the scientific enterprise in turning knowledge into useful products … so I came to branch out from what I was doing, to do something bigger and better, more intellectually interesting, and more practically important." Another commented: "I am sorta' on the fringe of science—but I am dealing with the core problems of society."

Our study supports the claim that "[b]right young scientists will gravitate toward the rich scientific opportunities at disciplinary boundaries" (*13*). It also suggests, however, that many still feel the tension between the scientific promise of the interdisciplinary path and the academic prospect of the tenure track.

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4. P. Weingart, N. Stehr, Eds. *Practising Interdisciplinarity* (Univ. of Toronto Press, Toronto, 2000).
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6. F. Kessel *et al.*, Eds., *Expanding the Boundaries of Health and Social Science: Case Studies in Interdisciplinary Innovation* (Social Science Research Council and Oxford Univ. Press, New York, 2003).
7. D. Stokols *et al.*, *Nicotine Tob. Res.* **5**, 21 (2003).
8. J. Cummings, S. Kiesler, *Soc. Stud. Sci.* (in press).
9. The five programs included a Human Dimensions of Global Change Center, two Integrative Graduate Education and Research Training programs, a National Synthesis Center, and a Science and Technology Center. See supplemental material for further information.
10. The affiliates across the five programs were distributed as 18% graduate students, 28% non–tenure-track faculty, 9% postdoctoral, 8% assistant professors, 9% associate professors, and 27% full professors (see the table); when the separate program percentages are averaged across the five programs, thus treating each program equally, the distributions are 32, 16, 6, 7, 9, and 28%, respectively. Both calculations point to light involvement of early career tenure-track faculty.
11. "Interdisciplinary" refers to relations that cross boundaries (e.g., engineering, physical science, life science, and social science).
12. D. Rhoten, A. Parker, unpublished data.
13. N. Sung *et al.*, *Science* **301**, 1485 (2003).
14. Supported by NSF grant BCS-0129573.

Supporting Online Material
www.sciencemag.org/cgi/content/full/306/5704/2046/DC1

10.1126/science.1103628

VIEWS ON CAREER EFFECTS OF INTERDISCIPLINARY RESEARCH

	Distribution by rank*							
	G	NTT	PD	AsP	AP	P	PIs	Total
Number surveyed	160	245	84	73	82	232	12	888
Total responses	99	155	59	47	53	147	11	571
Positive	67	104	42	34	43	109	11	413
Neutral	16	43	11	12	8	23	0	114
Negative	16	8	6	1	2	15	0	44

*G, graduate student; NTT, nontenure track; PD, postdoctoral fellow; AsP, assistant professor; AP, associate professor; P, professor; PI, principal investigator. [Source (*9*)]

ANTHROPOLOGY

The Astonishing Micropygmies

Jared Diamond

By now, every *Science* reader will have read about the discovery of skeletons representing a primitive human micropygmy population that survived until about 18,000 years ago on the Indonesian island of Flores (*1*, *2*). These creatures were barely 3 feet tall, and had an estimated body weight of 20 kg and a brain size of 380 cm^3 (smaller than that of a chimpanzee). They seem to be more similar to *Homo erectus* than to *Homo sapiens*, and are thought to have descended from *H. erectus* independently of *sapiens*' descent from *erectus*. When I first learned of this discovery, I thought it the most astonishing in any field of science within the last decade (see page 2013 of this issue). On reflection, paraphrasing Elizabeth Barrett Browning, let me count the ways in which it is (and is not) astonishing.

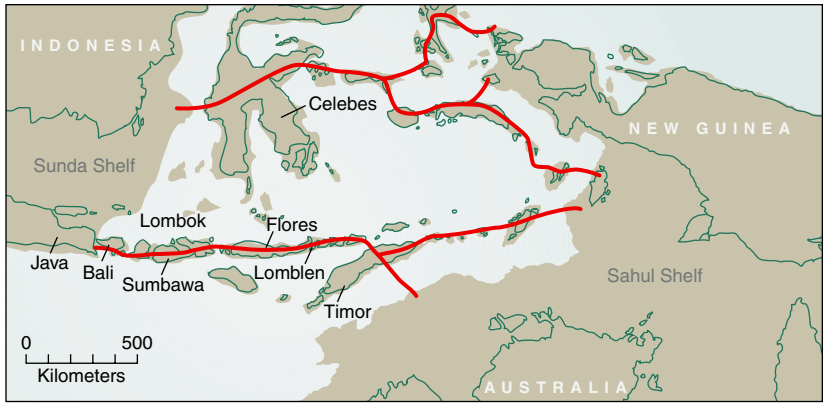
In situations like this one, I've found it useful to get the perspective of a green extraterrestrial friend visiting Earth from the Andromeda Nebula. My friend remarked, "Once again, you humans are prisoners of your ingrained species-centric biases. You already know that large mammals colonizing remote small islands tend to evolve into isolated populations of dwarfs. You have examples of insular pygmy hippos, buffalo, ground sloths, true elephants, stegodont elephants, mammoths, "Irish" elk, red deer, and even dinosaurs. So, now you have 10 examples instead of 9. What's so astonishing? Since when aren't humans subject to natural selection?"

E.T.'s response forced me to reflect. One surprise, I realized, is that we're uncertain exactly which selective pressures do select for insular dwarfs. A favorite theory is ecological release from competition, when a big species reaches an island lacking the mainland suite of smaller related species. According to this argument, the Flores micropygmy would have evolved to occupy a niche of abundant food left vacant by the lack of native apes, monkeys, and other small flightless mammals (except for rodents and a dwarfed elephant) on this island. Another favorite theory is the supposed resource poverty of islands, such that small-bodied animals will be less likely to starve than large-bodied animals. At the level of individual selection, that argument won't work: Flores and other islands with dwarfed mammals have productivities per hectare at least as high as those of continents. But the argument could work at the level of group selection and could explain the regularly increasing relation between body mass of an island's or continent's top carnivore (or herbivore) and the area of the land mass (*3*). What counts is the island's total productivity rather than its productivity per hectare: An isolated population of 100 full-sized human hunter-gatherers on Flores would have been at a much higher risk of extinction than an isolated population of 700 micropygmies.

E.T.'s blasé reaction then made me think further: Flores is just one of hundreds of islands in its size range, so why weren't there micropygmies on many other islands? The catch is that, for dwarfing to evolve on an island, you need humans just barely capable of reaching the island: If they could reach it too easily, the continuing arrival of full-sized colonists would prevent evolutionary divergence. Once modern *H. sapiens* developed the technology to reach islands, the resulting insular populations were constantly faced with new arrivals and were no longer isolated. Hence the only examples of effectively isolated insular *sapiens* populations known to me are from so-called land-bridge islands (like Britain and Japan) formerly connected to adjacent continents at Pleistocene times of low sea level, and isolated around 10,000 years ago when world ice sheets melted and sea levels rose. Some recent *sapiens* populations on those land-bridge islands were descended from ancestors who walked to the island during land-bridge times, lacked watercraft, and thus became completely isolated when the land bridge was severed.

For instance, the Australian land-bridge island of Tasmania is known to have supported a human population that survived in isolation for 10,000 years after Tasmania became cut off from Australia (*4*). Tasmania was large enough that full-sized humans are predicted from regression equations (*3*) to have lived there—and modern Aboriginal Tasmanians were indeed full-sized. However, the much smaller Australian land-bridge island of Flinders supported a human population that succumbed to isolation only after about 4000 years (*5*): I am unaware of skeletal remains that indicate whether these humans became reduced in size. Promising locations to search for *erectus* micropygmies are other Indonesian islands besides Flores: surely Lombok and Sumbawa, through which *erectus* colonists from the Asian mainland must have passed to reach Flores; and perhaps Sumba, Timor, Celebes, and others (see the figure). My first bet is on Celebes.

How did the ancestors of the Flores micropygmies, whoever they were, reach Flores? At Pleistocene times of low sea level, the Indonesian island chain of the Greater Sunda Islands was connected to



Island hopping in the Late Pleistocene. The island realm from Southeast Asia to Australia and New Guinea. Solid lines denote the current configuration of land. Brown shading denotes the configuration of land in the Late Pleistocene, when the sea level was about 150 m below its present stand, and when shallow seas on continental shelves now less than 150 m deep were dry land. At that time, Bali and Java were joined to each other and to the Asian mainland, Lombok was joined to Sumbawa, and Flores was joined to Lomblen. However, reaching Flores and Lomblen from Asia still required crossing three narrow water gaps, and reaching Australia from Timor or islands to the east would have required crossing even wider gaps of water. [Adapted from (*6*)]

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the Asian mainland as far east as Java and Bali, but water gaps of 6, 19, and 3 km, respectively, separated Bali from Penida, Penida from Lombok and Sumbawa (joined in the Pleistocene), and Lombok and Sumbawa from Flores and Lomblen (also joined in the Pleistocene) (*6*). Across each of those water gaps, the island on the far side would have been visible to someone standing on the island on the near side. Hence the micropygmies' ancestors could have colonized the island by sailing toward it in a watercraft (perhaps a rudimentary raft, or a mere floating log), or they could have landed on the island accidentally when their watercraft was swept to sea by ocean currents. Perhaps they even swam to the island. Stegodont elephants reached Flores and Timor and Celebes, and monkeys and buffalo and squirrels also reached Celebes, all surely without making rafts; *H. erectus* presumably could have as well.

Why haven't remains of *erectus*-like humans been found in Australia and New Guinea, at the eastern end of the Indonesian island chain? Possibly, for the same reason they weren't found on Flores until 2004; perhaps these humans did reach Australia and New Guinea, but archaeologists just haven't looked hard enough for their remains. I doubt this answer; hundreds of Pleistocene human sites are now known in Australia, with no remains of humans other than those of *sapiens*. Instead, the answer probably has to do with geography: A modern map plus bathymetric charts show that, even at Pleistocene times of low sea level, a water gap of at least 87 km separated the easternmost Indonesian islands from either Australia or New Guinea, which would not have been visible across that wide gap (*6*). Such gaps were too wide not only for pre-*sapiens* humans, but also for stegodonts, monkeys, buffalo, and squirrels, none of which are found in Australia and New Guinea.

The discoverers of the Flores micropygmies conclude that they survived on Flores until at least 18,000 years ago (*1*, *2*). To me, that is the most astonishing finding, even more astonishing than the micropygmies' existence. We know that full-sized *H. sapiens* reached Australia and New Guinea through Indonesia by 46,000 years ago, that most of the large mammals of Australia then promptly went extinct (probably in part exterminated by *H. sapiens*), and that the first arrival of behaviorally modern *H. sapiens* on all other islands and continents in the world was accompanied by similar waves of extinction/extermination. We also know that humans have exterminated competing humans even more assiduously than they have exterminated large nonhuman mammals. How could the micropygmies have survived the onslaught of *H. sapiens*?

One could perhaps seek a parallel in the peaceful modern coexistence of full-sized *sapiens* and pygmy *sapiens* in the Congo and Philippines, based on complementary economies, with pygmy hunter-gatherers trading forest products to full-sized *sapiens* farmers. But full-sized *sapiens* hunter-gatherers 18,000 years ago would have been much too similar economically to micropygmy hunter-gatherers to permit coexistence based on complementary economies and trade. One could also invoke the continued coexistence of chimpanzees and humans in Africa, based on chimps being economically too different from us to compete (very doubtful for micropygmies), and on chimps being too dangerous to be worth hunting (probably true for micropygmies). Then, one could point to the reported survival of the pygmy stegodont elephants on Flores until 12,000 years ago (*1*, *2*): If stegodonts survived so long in the presence of *H. sapiens*, why not micropygmies as well? Finally, one might suggest that all of the recent dates for stegodonts and micropygmies on Flores are in error [despite the evidence presented in (*1*) and (*2*)], and that both stegodonts and micropygmies became extinct 46,000 years ago within a century of *H. sapiens*' arrival on Flores. All of these analogies and suggestions strike me as implausible: I just can't conceive of a long temporal overlap of *sapiens* and *erectus*, and I am reluctant to believe that all of the dates in (*1*) and (*2*) are wrong. Hence I don't know what to make of the reported coexistence.

At last comes the question that all of us full-sized *sapiens* wanted to ask but didn't dare: Did full-sized *sapiens* have sex with micropygmies? The difference in body size would not have been an insuperable obstacle: Some individual modern humans have sex with children or with domestic animals no larger than the micropygmies. I suspect that the answer is the same as the answer to the question of whether we modern humans have sex with chimpanzees. We don't, because chimps are too unlike humans to appeal sexually to most of us, and because chimps are much too strong, unpredictable, and dangerous to make sex a safe proposition for any individual humans who might find them sexually attractive. Ditto for *H. erectus*, even when dwarfed.

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MATERIALS SCIENCE

Nucleic Acid Nanotechnology

Hao Yan

Nucleic acids are best known as the carriers of genetic information, but they are also a versatile material for designing nanometer-scale structures, because nucleic acid sequences can be designed such that the strands fold into well-defined secondary structures. In 1982, Seeman (*1*) first proposed using branched DNA building blocks to construct ordered arrays. In recent years, DNA has been shown to be an ideal molecule for building micrometer-scale arrays (*2*, *3*) with nanometer-scale features. DNA can also be used to make nanometer-scale materials with moving parts, such as nanotweezers (*4*).

Today, two major challenges face nucleic acid–based nanotechnology: to produce complex superstructures from simple molecular building blocks, and to perform controlled mechanical movements in molecular devices. Two reports in this issue describe steps to meet these challenges. On page 2072, Liao and Seeman (*5*) present a DNA device that can program the synthesis of linear polymers through positional alignment of reactants. And on page 2068, Chworos *et al.* (*6*) use rationally designed RNA building blocks as jigsaw puzzle pieces that direct pattern formation. The two studies demonstrate that it will be feasible to build functional materials and devices from "designer" nucleic acids.

Nanotechnology researchers have sought to mimic nature's biological motors to create nanometer-scale machines that can function in an engineered environment. Liao and Seeman take an important step in this direction with a device that mimics the translational capabilities of the ribosome. The device consists of two subsections, each with two structural states. Different pairs of DNA "set strands" can be added or removed to bring the device into any one of four states. Each state allows the positional

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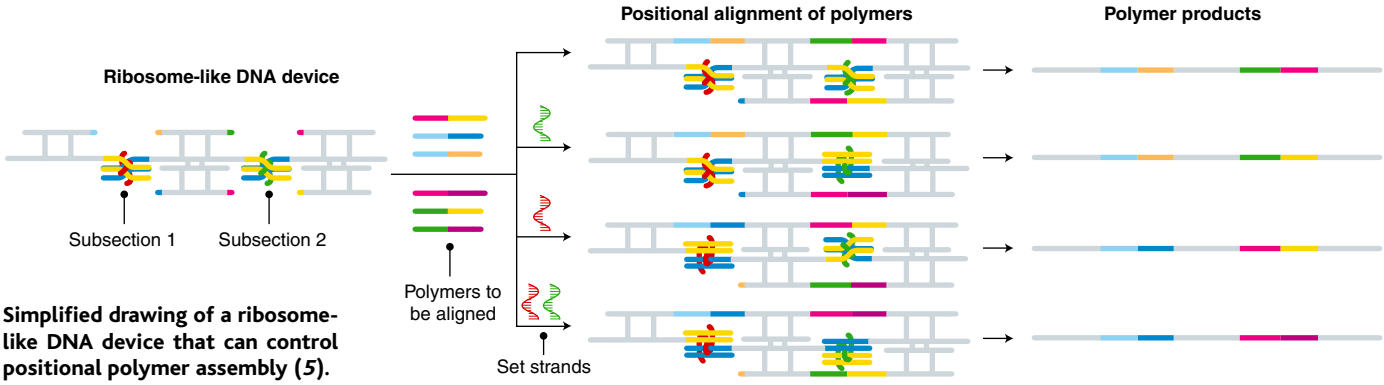


Simplified drawing of a ribosome-like DNA device that can control positional polymer assembly (*5*).

alignment of a specific pair of DNA motifs that are selected from a pool. The pairs bear polymer components that can then be fused in a specific order (see the first figure).

As proof of principle, Liao and Seeman use DNA as the polymer that is aligned, and enzymatic ligation to fuse the polymers. Positional synthesis with the prototype device thus results in four different DNA strands, each containing a defined sequence.

In this ribosome-like DNA device, there is no complementary relationship between the signal sequence and the products. Furthermore, all polymer reactants exist simultaneously in one solution. These features make the device appealing for building nanometer-scale machines that control massively parallel chemical synthesis. Liu and co-workers (*7*) have shown that DNA-templated organic synthesis can be used to discover new bond-forming chemical reactions.

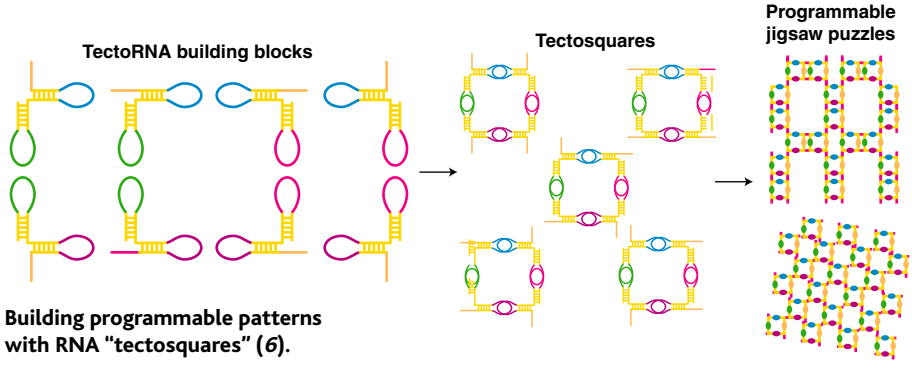
Future practical applications of nucleic acid–based nanotechnology will depend on our ability to design and self-assemble complex patterns efficiently. Chworos *et al.* describe exciting progress toward this goal. They have designed three-dimensional RNA building blocks that assemble into jigsaw puzzle pieces; the pieces then assemble further into a variety of two-dimensional, nanometer-scale structures with increasing complexity and addressability.

The jigsaw pieces (RNA tectosquares) each consist of four RNA building blocks (tectoRNA) (see the second figure) (*8*). Each tectoRNA contains two hairpin loops with a 90° angle between them; four tectoRNAs containing matching hairpin loop sequences can form a RNA tectosquare through noncovalent interactions between their loops. The authors use a pool of preformed tectosquares as modular building blocks that assemble into addressable patterns via sticky-tail connectors. The connectors are single-strand overhangs that protrude from one stem in each building block; by changing the sequence, the orientation of the tail can be varied without changing the positioning of the stem-loop arms (see the second figure). Furthermore, tectosquares of different sizes can be constructed by using hairpin stems of different lengths, thus providing additional degrees of freedom for designing the modular building blocks.

Chworos *et al.* have synthesized 49 tectoRNAs with different sizes, tail sequences, tail lengths, and tail orientations. They combined them to construct 22 tectosquares that were subsequently mixed to generate nine different, predefined, finite, and periodic patterns.

RNA tectosquares provide a new toolbox for nucleic acid–based nanotechnology. How complex can the patterns be? In 1996, Winfree (*9*) proposed that if self-assembly proceeds by cooperative binding at multiple weakly binding domains, it should be possible to encode any desired algorithmic rules in a set of "molecular tiles" (such as the RNA tectosquares) that will self-assemble into a potentially quite complex pattern. Indeed, Rothemund *et al.* (*10*) have recently used algorithmic DNA self-assembly to construct a fractal pattern referred to as a Sierpinski triangle; this work demonstrates that engineered DNA self-assembly can be treated as a Turing universal biomolecular system (Turing universal computing is a form of computing that can emulate any other computing method).

Further progress in constructing molecular devices and patterned superstructures based on nucleic acids will require methods to reduce errors in self-assembly, to template functional nanoelectronics on nanometer-scale DNA fabrics, to extend two-dimensional self-assembly to three dimensions, and to scale up self-assembly. Recent progress on error-correcting mechanisms (*11*), molecular lithography based on DNA-based nanometer-scale assemblies (*12*, *13*), DNA-templated metallic nanoparticle arrays (*14*), and a replicable, three-dimensional, nanometer-scale DNA octahedron (*15*) promises an exciting future for nucleic acid nanotechnology.



Building programmable patterns with RNA "tectosquares" (*6*).

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Oxygen Sensing: It's a Gas!

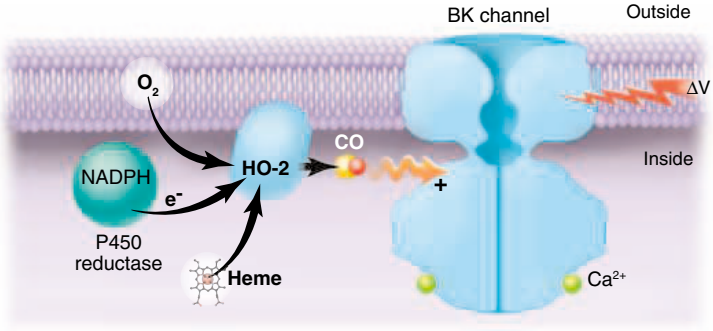
Toshinori Hoshi and Sukhamay Lahiri

Animals require oxygen to survive, and have evolved different mechanisms to sense and respond to low oxygen tensions. When faced with low blood oxygen levels (hypoxia), humans and other mammals reflexively increase the lung ventilation rate to restore normal oxygen tensions to vital organs. This compensatory mechanism relies on oxygen-sensing glomus cells in the carotid body located in the carotid artery. Glomus cells sense hypoxia and respond by rapid membrane depolarization. This results in production of action potentials, influx of calcium ions (Ca^{2+}) into the glomus cells through voltage-gated Ca^{2+} channels, and release of the neurotransmitter dopamine (*1*, *2*). Dopamine then activates postsynaptic sensory neurons, and this afferent discharge initiates a variety of responses by the central nervous system that ensure appropriate oxygenation of different organs. But how does hypoxia induce the rapid depolarization of glomus cells? On page 2093 of this issue, Williams *et al*. (*3*) answer three key questions that have kept investigators guessing: What is the oxygen sensor? What is the effector in glomus cells responsible for their depolarization? What is the messenger that couples the oxygen sensor to the effector?

Glomus cells are equipped with a full complement of ion channels, any of which could mediate rapid depolarization in response to hypoxia. Evidence suggests that many of these channels are regulated by hypoxia to different degrees, rendering a number of them potential oxygen-sensing effectors. Among them, however, is a large-conductance Ca^{2+}- and voltage-gated potassium (BK) channel that may be particularly important. BK channels have extraordinarily large conductances, >200 pS under some conditions. They are allosterically activated by intracellular Ca^{2+} ions and membrane depolarization. Under normal conditions, some BK channels are open and thus exert a negative influence on cell excitability by keeping the membrane in a hyperpolarized state (*4*). Pharmacological blockers of BK channel activity induce depolarization similar to that observed with hypoxia (*1*, *5*). Williams and colleagues performed electrophysiological experiments on native glomus cell BK channels as well as BK channels expressed in cultured mammalian cells (heterologous expression). They discovered that low oxygen tensions result in closure of the BK channels near the resting potential in excised patches of plasma membrane largely devoid of cytoplasmic components (*6*, *7*). This finding establishes BK channels as an important effector of the oxygen-sensing process in glomus cells.

What is the oxygen sensor in glomus cells and what couples the oxygen sensor to the BK channel effector? The observation that the inhibition of BK channels induced by hypoxia persists in excised membrane patches is crucial. This indicates that an intact cytoplasmic signaling cascade network is not essential, and that cellular constituents near the BK channel are sufficient for hypoxia-mediated inhibition of channel activity. Thus, the oxygen sensor that triggers BK channel inhibition must reside near the channel—a signaling molecule could readily travel from the sensor to the channel. A recent study (*3*) showed that the BK channel's sensitivity to hypoxia may be mediated by another gas, carbon monoxide (CO), and that the underlying mechanism may involve an increasingly common principle in cellular signal transduction: a spatially tuned local signaling pathway mediated by a large protein complex (*8*, *9*).

Numerous ideas about the nature of the oxygen sensor and the coupling molecule have been proposed. They generally fall into two closely related classes: the redox hypothesis and the heme-protein hypothesis. The redox hypothesis postulates that changes in oxygen tension may alter production of intracellular reactive species by mitochondria or by NADPH oxidases (NADPH is the reduced form of nicotinamide adenine dinucleotide phosphate), and that the subsequent redox change inhibits BK channel activity. Although somewhat counterintuitive, hypoxia may transiently increase the production of such reactive species (*10*, *11*). Furthermore, many experiments have shown that oxidation generally inhibits BK channel activity (*12*–*14*). The heme-protein hypothesis, on the other hand, suggests that binding of oxygen to (or release of oxygen from) heme-containing proteins forms the oxygen sensor. Many heme proteins are gas-sensitive, as exemplified by binding of oxygen to hemoglobin and myoglobin, of nitric oxide (NO) to guanylate cyclases, and of CO to cytochrome oxidases. Consistent with the heme-protein hypothesis, CO-mediated inhibition of cytochrome oxidases in mitochondria antagonizes most of the hypoxia sensitivity of glomus cells. However, the physical link between inhibition by CO and inhibition of plasma-membrane channels remained unclear (*15*).



Oxygen sensations. In response to a decrease in oxygen tension (hypoxia), BK channel activity is blocked and the channel closes, leading to depolarization of glomus cells in the carotid body. Oxygen sensing depends on the cooperation of BK channels with hemoxygenase-2 (HO-2) and carbon monoxide (CO). In the presence of oxygen, HO-2 uses electrons from NADPH cytochrome-P450 reductase and intracellular heme to generate CO, which in turn exerts a tonic excitatory influence on neighboring BK channels, which remain open. Hypoxia disrupts the production of CO and consequently inhibits BK channel activity. Only two of the multiple Ca^{2+} binding sites of BK channels are shown. It is not known how many CO molecules are required to stimulate BK channel activity. HO-2 may interact directly with the BK channel.

The study by Williams *et al*. (*3*) now links BK channel inhibition to heme in a potentially physically intimate way. The authors were motivated by the idea that a protein near the BK channel must be the oxygen sensor (because hypoxia-induced inhibition of BK channels persists in excised membrane patches). Using a proteomics approach, they found that the BK channel pore-forming α subunit coimmunoprecipitated with hemoxygenase-2 (HO-2). The potential colocalization of these two proteins was also implied by immunocytochemical analysis and confocal microscopy.

HO-2 is one of the two major forms of the enzyme hemoxygenase (HO). HO is a membrane-bound enzyme that oxidatively breaks down heme to CO, biliverdin, and iron. This reaction requires oxygen and uses NADPH–cytochrome P450 reductase as the

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electron donor protein. The potential colocalization of HO-2 with the BK channel and the characteristics of the heme breakdown reaction—oxygen requirement and CO generation—render HO-2 and CO prime candidates for the oxygen sensor and coupling messenger, respectively. CO, an emerging gaseous messenger akin to NO, is known to stimulate opening of BK channels (*16*–*18*), making the idea even more attractive. Thus, Williams *et al*. hypothesized that in the presence of oxygen, HO-2 could break down heme and so generate CO, which in turn would keep nearby BK channels open (see the figure). Hypoxia interferes with heme breakdown and CO generation, thus inhibiting channel opening.

Williams and colleagues showed that a CO donor increased the probability that BK channels—comprising the pore-forming α subunit and the auxiliary β_1 subunit—expressed in cultured mammalian cells remain open (*3*). In contrast, biliverdin, another product of the heme breakdown reaction, had no effect on the opening of BK channels. Addition of exogenous heme and NADPH, both of which are required to drive the HO-2 catalytic cycle to generate CO, also increased BK channel activity, whereas hypoxia effectively decreased channel activity. To show that hypoxia inhibits the reaction mediated by HO-2, the authors reduced HO-2 expression using a small interfering RNA (siRNA). As predicted by the HO-2 hypothesis, gene silencing of HO-2 by the siRNA essentially eliminated hypoxic inhibition of heterologously expressed BK channels. Importantly, the hypoxia sensitivity of native BK channels in glomus cells was enhanced by heme and NADPH, suggesting that the HO-2–based mechanism is operative in vivo.

The molecular scenario envisioned by Williams *et al*. places the HO-2 protein and the BK channel complex in close proximity. The breakdown of heme generates CO, which diffuses toward neighboring BK channels, keeping them open. Inhibition of this reaction by lack of oxygen interferes with the production of CO and so the BK channel closes, leading to depolarization of the glomus cell membrane. Although elegant, this scenario does not answer several important questions. Do HO-2 and BK channel complexes interact physically and directly, or are they simply accidental neighbors? The mechanism by which CO activates BK channels needs further investigation. Evidence suggests that CO may alter BK channel activity in a Ca^{2+}-dependent manner (*6*, *19*), but the details are sketchy. To establish further physiological relevance, it will be necessary to examine whether oxygen sensing is altered in mice that lack either the α or β_1 subunits of the BK channel (*20*–*23*).

Is this HO-2/CO-based mechanism of oxygen sensing universal? The answer appears to be no with respect to both the effector channel type and the signaling molecule. Numerous other channels are regulated by hypoxia, and a diverse class of signaling molecules has been implicated (*24*, *25*). The transcription factors hypoxia-inducible factor 1α (HIF-1α) and HIF-1β are likely to be important in genomic responses to chronic hypoxia (*26*). It is also possible that hydroxylation of HIF-1α may contribute to depolarization of glomus cells in response to hypoxia (*27*).

An oxygen-sensing mechanism incorporating a proximal HO-2 enzyme and the BK channel is likely to be one of many strategies that cells commandeer to respond to hypoxia. Nevertheless, Williams *et al*. clearly establish the importance of the HO-2 enzyme and the gaseous messenger CO as the oxygen-sensing mechanism of glomus cells. Other work has established free heme as an important regulator of BK channel activity (*28*). Further exploration of the functional and physical coupling of HO-2 and BK channels should be a gas!

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CELL BIOLOGY

"Pumping" Iron: The Proteins

Ernest Beutler

Thanks to its unique chemical properties, iron plays a central role in biology. Although iron is vital for life, it is highly reactive and so can be toxic when in excess. Hence, evolution has developed mechanisms to regulate the amount of iron in the cells of our body. Painstaking studies of iron balance in humans 65 years ago showed that virtually no iron is excreted and that stable iron levels are maintained by modulating absorption of iron from the gut (*1*). Iron homeostasis is complex, as there are many different proteins that respond not only to the total body burden of iron, but also to stimuli such as hypoxia, anemia, and inflammation.

There are two very different aspects to iron homeostasis. First, iron modulates the synthesis of a variety of proteins involved in iron metabolism, including the iron storage protein ferritin, the iron transporter transferrin, and the transferrin receptor. Second, another group of proteins regulates the transport of iron into and out of cells. In response to iron deficiency, hypoxia, or anemia, more iron is transported out of the gut lumen into intestinal epithelial cells, and then from the intestinal epithelia and liver macrophages (in the form of iron recycled from hemoglobin) into the blood. Inflammation and iron overload have the opposite effect, decreasing the amount of iron absorbed from the gut and released into the blood. The regulation of iron metabolism proteins by iron and the control of iron transport are undoubtedly connected, but how? Two reports on pages 2087 (*2*) and 2090 (*3*) of this issue shed new light on these processes.

More than 15 years ago, elegant studies by Hentze, Rouault, Klausner, and their associates established the existence of RNA motifs called iron responsive elements (IRE) in numerous transcripts of genes involved in iron metabolism and homeostasis. These motifs are bound by iron regulatory proteins 1 and 2 (IRP1 and IRP2) depending on cellular iron levels [reviewed in (*4*–*6*)]. When these proteins bind to IRE motifs in the 5′-untranslated region of, for example, the ferritin mRNA transcript, translation of the transcript is blocked and synthesis of ferritin is halted. In contrast,

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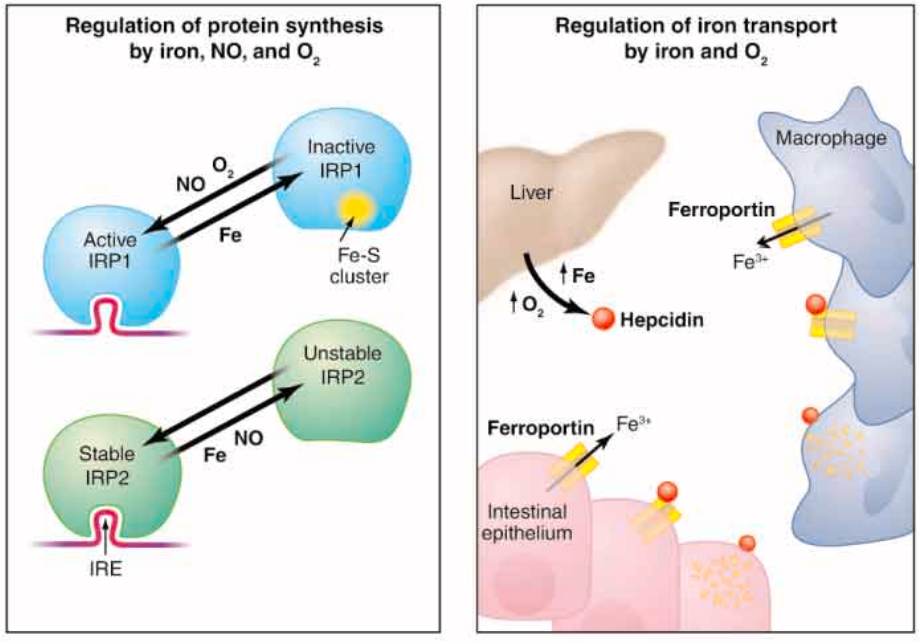
when IRP1 and IRP2 bind to the IRE in the 3′-untranslated region of, for example, the transferrin receptor transcript, the transcript is stabilized, translation proceeds, and the transferrin receptor is synthesized. Evolution has generously provided two IRPs, both of which bind to IREs but sense iron in very different ways (see the figure). IRP1 is a bifunctional cytosolic protein that contains an iron-sulfur cluster. In the presence of iron, IRP1 acts as an aconitase (interconverting citrate and isocitrate), but in the absence of iron, IRP1 binds to the IREs of various iron homeostasis transcripts with high affinity. By contrast, IRP2 undergoes iron-dependent degradation in iron-replete cells and therefore is not available to bind to the IREs. But things are a little more complicated than this. IRP2 is also sensitive to degradation in the presence of nitric oxide (NO), whereas IRP1 is activated by NO (*2*). It had been presumed that IRP1 is the principal iron sensor and a major player in iron homeostasis, yet mice deficient in IRP1 appear normal. In contrast, mice deficient in IRP2 show pronounced misregulation of iron metabolism and nerve damage.

In the new work, Rouault's group (*2*) provides some answers. These investigators show that cells from IRP2-deficient mice are unable to regulate iron when cultured in 3 to 6% oxygen (the physiological oxygen concentration in tissue), but could do so in 21% oxygen because IRP1 operates as an iron sensor only in a high-oxygen environment. Thus, IRP2 is the predominant regulator of iron homeostasis in mammalian cells at physiological oxygen tensions, and IRP1 acts as both an oxygen sensor and an iron sensor. These authors suggest that destabilization of IRP1 when bound to iron also occurs during inflammation through the action of oxygen free radicals. This interaction results in increased expression of the transferrin receptor and decreased expression of ferritin.

Inborn errors of metabolism provide some of the best insights into physiological pathways. Hence, identification of the mutations causing hereditary hemochromatosis—a disease characterized by excessive absorption of iron from the gut—was expected to garner new insights into the regulation of iron homeostasis. But surprisingly, the hemochromatosis gene (*HFE*) proved to encode not a protein involved in iron homeostasis but a major histocompatibility complex class I antigen. The discovery of the hemochromatosis gene shed no light on iron homeostasis whatsoever; it merely deepened the mystery. It would have been reasonable to suppose that the iron-oxygen-NO sensor (the whole IRP/IRE system) that serves so well to make iron-dependent changes in protein synthesis would also be a key player in iron transport. Yet *HFE* mRNA transcripts do not contain IRE elements and so presumably are not bound by either IRP1 or IRP2. Mutations in other genes—such as those encoding β_2-microglobulin, hemojuvelin, hepcidin, ferroportin, transferrin receptor–2, and transferrin [see recent reviews (*7*, *8*)]—also result in iron overload in both humans and mice. Furthermore, mutations in genes encoding hephaestin and the divalent metal transporter–1 (DMT1) result in iron deficiency. Despite the fact that these proteins are all involved in maintaining appropriate iron levels in the body, our understanding of how these gene products modulate iron transport is fragmentary. Hepcidin is a peptide of 25 amino acids that is secreted by the liver in response to iron overload and inflammation. It is regarded as a central regulator of iron homeostasis because mutant forms of this protein are associated with particularly severe iron overload. Moreover, mutant forms of HFE, transferrin receptor–2, and hemojuvelin result in a decreased hepcidin response to an increased iron load, which suggests that hepcidin synthesis is regulated by these proteins (*9*). Apart from these fragmentary findings, however, nothing is known about how hepcidin is involved in iron homeostasis.

Enter Nemeth and her colleagues (*3*) with their new study, which provides some pieces to the puzzle. Their findings spotlight the importance of ferroportin, an iron exporter protein expressed on the surface of gut enterocytes, macrophages, and liver cells. Indeed, ferroportin has characteristics that set it apart from other proteins involved in iron homeostasis. Among proteins that when defective cause iron overload or iron deficiency, only ferroportin and DMT1 contain IREs, and only mutations in the ferroportin gene are inherited in an autosomal dominant fashion. This implies that ferroportin is a rate-limiting factor in iron absorption and that ferroportin may be a key regulator of iron transport. But why would decreasing the amount of ferroportin in the abluminal membrane of intestinal mucosal cells result in excess iron accumulation? Indeed, the prediction would be that fewer channels for iron transport would result in iron deficiency. In the zebrafish, where this mutation was first discovered (*10*), the phenotype is called weissherbst (a type of white wine) because the fish exhibit iron deficiency anemia. With their demonstration that



Regulation of iron homeostasis. (Left) IRP1 and IRP2 regulate the synthesis of proteins involved in iron homeostasis by binding to RNA motifs (IREs) in the mRNAs of these proteins. When iron is present, IRP1 does not bind to the IRE, but when iron is removed, IRP1 binds to the RNA motif and regulates protein synthesis. IRP2, the more important regulator of iron homeostasis in mammalian cells, is degraded in the presence of iron but binds to the IREs in the absence of iron (*2*). Both IRP1 and IRP2 are also regulated by nitric oxide but in opposite ways; IRP1 is also an oxygen sensor, binding to IREs in response to high oxygen tensions. **(Right)** Hepcidin, secreted by liver cells in response to iron overload or inflammation, regulates the transport of iron out of cells. It does this by binding to the iron exporter ferroportin expressed on the surface of gut enterocytes. This interaction results in the internalization of ferroportin and its destruction, leading to a decrease in iron transport out of macrophages and intestinal mucosal cells into the blood and a lowering of plasma iron levels (*3*).

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ferroportin interacts with hepcidin, Nemeth *et al*. provide an explanation. They demonstrate in tissue culture cells that hepcidin binds to ferroportin, which results in internalization and degradation of ferroportin leading to decreased export of cellular iron. The authors propose that this posttranslational regulation of ferroportin activity by hepcidin completes a homeostatic loop whereby iron regulates the secretion of hepcidin by liver cells, and then hepcidin controls expression of ferroportin on the surface of gut enterocytes, which then reduces export of cellular iron.

Although tantalizing, other recent observations suggest that this model does not tell the whole story. A mouse in which the 5′ IRE, a negative regulatory element, is deleted does not manifest an increased iron burden, but surprisingly exhibits a complex phenotype: iron deficiency at birth with elevation of body iron levels only later in life and eventual normalization of iron stores. Mice heterozygous for deletion of the 5′ IRE manifest a marked increase in red blood cell production 7 weeks after birth due to increased transcription of the gene encoding the hematopoietic growth factor erythropoietin. These findings imply strong links among the pathways that regulate red blood cell production and iron homeostasis. For example, it would not be surprising if HIF-1α—a transcription factor that switches on genes including the erythropoietin gene in response to hypoxia—is involved in iron homeostasis.

Despite these complications, the Nemeth *et al*. study does much to clarify how the efferent branch of iron regulation works. Liver cells are stimulated directly to increase transcription of the hepcidin gene by inflammatory cytokines and by rising oxygen tensions, but not by addition of iron or iron-laden macrophages to cultured liver cells. How, then, does the body signal liver cells to augment transcription of the hepcidin gene when iron levels increase? And why is activation of hepcidin gene expression by iron loading suboptimal in the absence of HFE, transferrin receptor–2, or hemojuvelin? Do these proteins all reside on the same signaling pathway or are they components of several different signaling pathways? And which is the actual pathway that transmits the signal to liver cells to boost production of hepcidin in response to hypoxia, anemia, inflammation, and iron overload? The new findings reported in this issue have put some of the puzzling pieces about iron regulation into place, but a full understanding of the complicated interrelationships among pathways of iron homeostasis still eludes our grasp.

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SIGNAL TRANSDUCTION

Unexpected Mediators of Protein Phosphorylation

John D. York and Tony Hunter

A decade ago, Snyder postulated that a newly discovered class of high-energy molecules called pyrophosphorylated inositol polyphosphates (PP-IPs) could donate phosphate groups to proteins. On page 2101 of this issue, Saiardi *et al*. (*1*) report evidence to support this intriguing notion. That inositol phosphate signaling molecules can directly mediate protein phosphorylation, usually the domain of the huge kinase family, represents a new way in which signals can be transduced in cells. Cellular regulation by soluble inositol phosphate messengers continues to yield surprises that reach well beyond the rigorously characterized regulation of calcium release by inositol 1,4,5-trisphosphate (IP_3), the best known inositol phosphate.

The PP-IPs are a distinct class of inositol phosphates that were first identified in slime molds (*2*) and later in mammalian cell extracts treated with fluoride (*3*). Paradoxically, these inositides appeared to be more polar than inositol hexakisphosphate (IP_6), which was then believed to be the fully phosphorylated form of *myo*-inositol (the precursor of all inositol phosphates). Subsequently, two groups (*3*, *4*) identified these molecules as PP-IPs. The next breakthrough came with the identification of the IP_6 kinase (designated IP6K), which catalyzes the production of PP-IPs (*5*). The synthesis of PP-IPs also requires phospholipase C activity and the subsequent conversion of released soluble IP_3 to inositol 1,4,5,6-tetrakisphosphate (IP_4), inositol 1,3,4,5,6-pentakisphosphate (IP_5), and IP_6 through the action of inositol polyphosphate kinases (IPKs) (*6*). IP6Ks are members of a small family of evolutionarily conserved IPKs defined in part by a Pro-X-X-X-Asp-X-Lys-X-Gly signature sequence (*7*, *8*). Loss of IP6K activity results in many different cellular defects, including aberrant DNA recombination, and abnormalities in vacuolar morphology, gene expression, chemotaxis, osmotic stress, and telomere length (*9*–*14*). These findings, coupled with recent genetic and biochemical studies of the IPKs in a variety of eukaryotic cells, have invigorated research in this field and unraveled roles for these regulators in diverse areas of biology, including mRNA export, transcription, chromatin remodeling, DNA metabolism, telomere maintenance, and membrane trafficking (*7*, *8*, *14*).

Taking a biochemical approach, Snyder, Saiardi, and their colleagues now show that the product of IP6K, PP-IP_5 (also called IP_7), nonenzymatically donates a phosphate group to specific protein substrates in a magnesium-dependent manner, in the absence of any known protein kinase (*1*). Through elegant experiments, these authors show that the β-position phosphate is the donor and that, intriguingly, one of the relevant substrates is Nsr1, the budding yeast version of nucleolin (a protein involved in ribosome biogenesis). More than one amino acid residue in a serine-rich region flanked by acidic residues near the amino terminus of Nsr1 is phosphorylated. It appears that the substrate protein must be "primed" by the cell, because recombinant Nsr1 produced in bacteria does not accept a phosphate group from PP-IP_5 (*15*). Such priming may occur through posttranslational modification, for example, phosphorylation of Nsr1 by a kinase. Total Nsr1 phosphorylation was reduced in yeast cells lacking IP6K activity, consistent with the physiological importance of PP-IP_5.

Perhaps the most exciting aspect of the new work is that it defines a new mechanism in second messenger biology (see the

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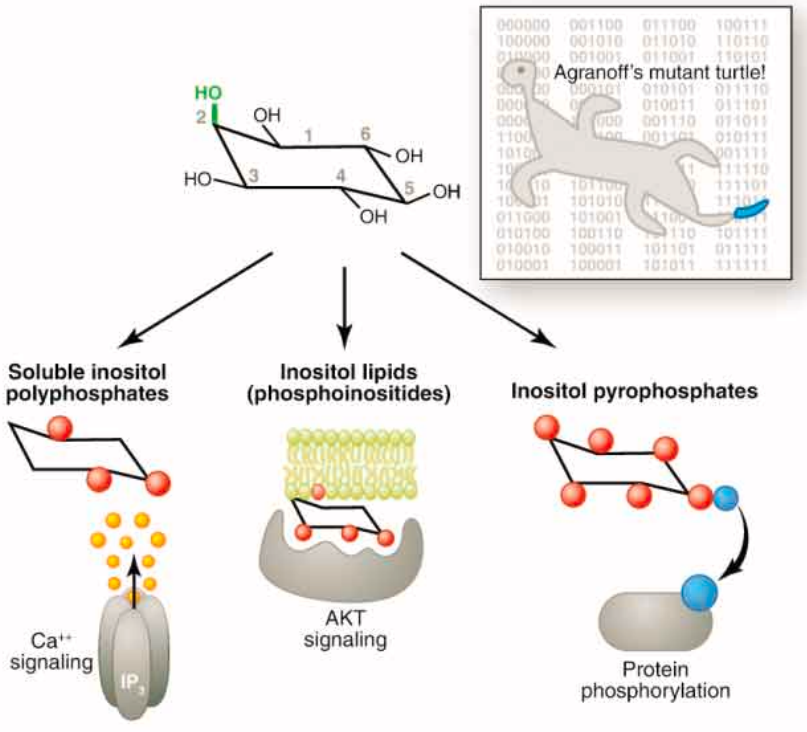
figure). Up until now, two general modes of signaling mediated by inositides have been described. Water-soluble inositol polyphosphates use a classic mode of signal transduction that depends on transient binding to a substrate and a subsequent conformational change in the effector—for example, binding of IP_3 to the tetrameric calcium channel receptor and allosteric gating of calcium ion release. Although other intracellular second messengers, such as adenosine 3′,5′-monophosphate (cAMP), behave in a similar way, the exquisite specificity of the IP_3 receptor and the fact that IP_3 serves as a precursor to more than 20 other IPs (such as IP_4, IP_5, and IP_6) make soluble inositol polyphosphates unique players in cell biology. A second mode of signal transduction occurs through membrane signaling involving the inositol phosphate head group linked to a glycerophospholipid backbone. The discovery of phosphatidylinositol 3-kinase (PI3K) prompted the idea that inositol lipids serve as second messengers in their own right. To this end, their synthesis in cellular membranes triggers the recruitment of signaling proteins such as the serine-threonine kinase Akt through 3′-phosphoinositide binding domains. Phosphoinositides are also crucial in the regulation of membrane trafficking, rearrangements of the actin cytoskeleton, and cell motility. The Saiardi *et al*. study now defines a third mode of inositide-mediated signaling through direct transfer of phosphate groups from PP-IPs to protein substrates (*1*). The high-energy phosphodiester bond donates a phosphate to primed protein substrates such as Nsr1, providing a new mechanism for protein phosphorylation (see the figure).

The Saiardi *et al*. data raise interesting questions about how the PP-IP acts as a phosphate donor. What is the specificity and stereochemistry of PP-IP binding? How is phosphate transfer activated? How is a target serine/phosphoserine selected? The exact nature of the peptide modification and the physiological relevance of the process is also not known. Given the nonenzymatic but highly selective nature of the PP-IP–catalyzed reaction, it is possible that the peptide substrate conformation aids in the reaction, acting as a "transferase." Mutations in the surrounding acidic and basic residues that ablated Nsr1 phosphorylation by $PP\text{-}IP_5$ support this conclusion. However, the fact that the application of heat enhanced the efficiency of phosphorylation confounds this interpretation. Although the investigators deduced that $PP\text{-}IP_5$ modifies serine residues, it is surprising that they did not report conventional phosphoamino acid analysis—for example, through total acid/proteolytic digestion. Snyder has indicated that these experiments were performed but were not definitive (*15*). This result, coupled with an apparent requirement for priming the substrate (possibly by prephosphorylation), suggests that the product could be a pyrophosphorylated serine. Precise determination of the nature of the modification and of the identity of the residues phosphorylated in vitro awaits mass spectrometry and structural analyses. This information is a prerequisite for verifying the underlying assumption that PP-IPs phosphorylate Nsr1 and other targets in vivo, and also for establishing that PP-IP–mediated phosphorylation regulates the function of phosphorylated targets. Also, the extent of $PP\text{-}IP_5$–mediated protein phosphorylation and the total number of cellular substrates for this reaction need to be determined. In the Saiardi *et al*. study, Srp40 and Ygr130 were also identified as protein substrates of $PP\text{-}IP_5$–mediated phosphorylation. The physiological relevance of PP-IP phosphorylation remains an open question. Although Nsr1 phosphorylation in yeast cells may be altered through changes in IP6K activity, it is not clear whether this is a direct effect or whether this modification alters the biological function of Nsr1. Given that Nsr1 may be involved in a number of cellular processes, including ribosome biogenesis, it will be exciting to probe whether IP6K and $PP\text{-}IP_5$ are involved in this process. Conversely, given the myriad tasks already discovered for IP6Ks, the next step will be to determine whether any of these functions requires phosphorylation mediated by $PP\text{-}IP_4$ or $PP\text{-}IP_5$.

The field of inositide regulation was launched more than 50 years ago when a dedicated duo of scientists carried extracts of agonist-stimulated pigeon pancreas across the Atlantic. Since then, the field has encountered many milestones: The discovery by Saiardi *et al*. that inositol polyphosphates are capable of phosphorylating proteins is sure to figure prominently among them. One might ask "Why inositol polyphosphates?" The answer may lie in looking at the chemistry of *myo*-inositol (the precursor of inositol polyphosphates) and at modern computer hardware (see the figure). Nature may have chosen inositol polyphosphates because they represent an elegant biological signal-



Three's not a crowd. Three inositide signaling pathways are shown. The first is allosteric regulation as exemplified by IP_3, which binds to calcium channels and alters their conformation resulting in release of calcium ions (yellow dots). The second is membrane recruitment exemplified by translocation of the serine/threonine kinase Akt by inositol lipids; here, the lipid is phosphatidylinositol 3,4,5-trisphosphate (PIP_3). The third is protein phosphorylation by the inositol polyphosphate $PP\text{-}IP_5$, which donates a β-phosphate to proteins such as Nsr1 in yeast and nucleolin (*1*). (**Inset**) The chemistry of *myo*-inositol, the precursor of inositol polyphosphates. Depicted is *myo*-inositol in the form of Agranoff's mutant turtle with two tails (*16*). The head represents the axial D-2 position hydroxyl; the flippers and tail represent the five equatorial hydroxyl groups. Duplication of the tail represents the pyrophosphate group (blue). The concept of a 6-bit signaling chip is illustrated by a binary code representation of the 64 theoretical combinations of inositides. Phosphate groups are represented by the red and blue dots.

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ing "chip" that enables cells to generate vast, combinatorially complex arrays of communication pathways.

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CHEMISTRY

Whence Molecular Electronics?

Amar H. Flood, J. Fraser Stoddart, David W. Steuerman, James R. Heath

The drive toward yet further miniaturization of silicon-based electronics has led to a revival of efforts to build devices with molecular-scale components. The field of molecular electronics is teeming with results, rationalizations, and speculations. Some claims may have been exaggerated, but news stories of a crisis in the field (*1*) are premature. Reports of passive molecular electronics devices, such as tunnel junctions and rectifiers, as well as of active devices, for example, single-molecule transistors and molecular switch tunnel junctions, have withstood scientific scrutiny. Simple molecular electronic devices usually consist of organic molecules sandwiched between conducting electrodes. According to early predictions, such devices could show electron tunneling (*2*) or one-way flow of current (rectification) through the molecule (*3*). In most tunneling junctions, linear alkanes are sandwiched between metal electrodes. Measurements over the past 25 years (*4*, *5*) have largely validated McConnell's prediction (*2*) that the tunnel current depends exponentially on the length of the molecules between conducting electrodes. In rectifiers, a molecule composed of an electron donor, a bridge, and an electron acceptor is extended between two electrodes (see the first figure, top panel). Experiments (*6*, *7*) have again validated the early prediction by Aviram and Ratner (*3*).

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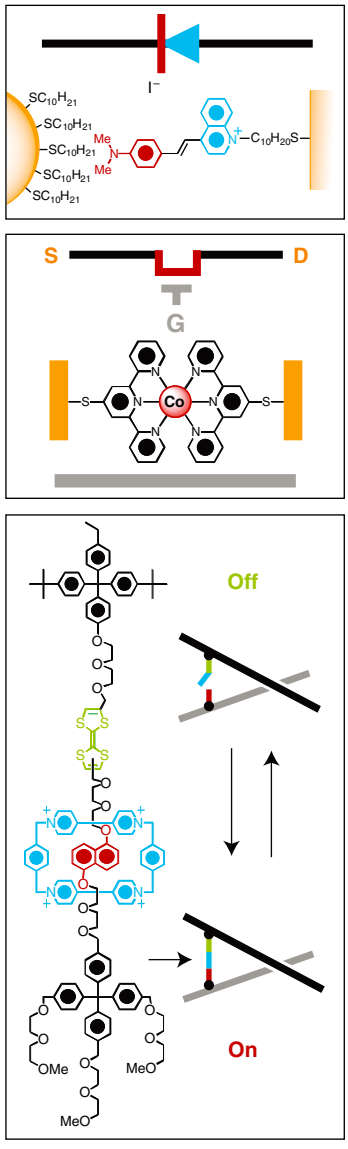
However, for both types of devices, the devil has been in the details, and reaching agreement between experiments and theory has not been straightforward. In the case of tunnel junctions, McConnell's prediction breaks down for alkanes with more than about 16 carbon atoms in the chain, because coherent tunneling is replaced by diffusive charge transport in longer chains. Furthermore, in all devices, the molecules tilt at an angle smaller than 90° with respect to the electrode surfaces. This angle—and hence the separation between the electrodes—varies across different device constructions. Such variations can affect the measured current levels and can also dictate at which alkane chain length diffusive transport replaces tunneling. Other issues, such as the choice of electrode materials, can have similar effects.

In the case of rectifiers, it has turned out to be relatively easy to observe rectification, but nontrivial to observe true molecular rectification. This problem arises because current can be rectified in many parts of the device—for example, at the molecule/electrode interfaces. True molecular rectification is observed only if the donor–bridge–acceptor component of the molecule is extended between the electrodes, and for only a relatively small range of donor and acceptor molecular orbital energy levels. Thus, strict attention to the molecular components, and to the molecule/electrode interfaces, is required.

Active molecular electronic devices include single-molecule transistors and molecular switch tunnel junctions. The development of these more complex devices has been guided by experiment rather than theory. To date, only a couple of systems have passed scientific scrutiny from multiple laboratories. To validate such devices, one compelling approach has been to identify unique properties that can be observed and quantified in both the devices and in solution.

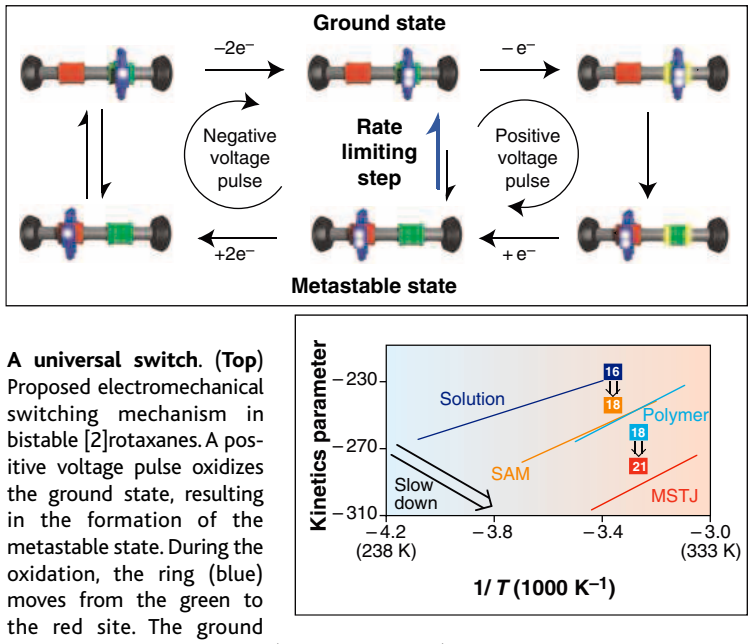
In a single-molecule transistor, a molecule is bridged across a 1- to 4-nm-wide electrode gap (see the first figure, middle panel). Three-terminal devices of this kind are powerful tools for exploring the fundamental physics of molecular devices: Parameters such as temperature, and electric and magnetic fields, may all be varied while the spectroscopic response is measured. Using single-molecule



Molecular electronic devices. (**Top**) A molecular rectifier constructed from donor (red), bridge (black), and acceptor (blue) components. (**Middle**) A single-molecule transistor constructed from a symmetrical cobalt complex. S, source; D, drain; G, gate. (**Bottom**) A molecular switch tunnel junction in its Off and On states. (Left) Structural formula of the On state of a bistable [2]rotaxane.

transistors, two groups (*8*, *9*) have observed a unique type of quantum mechanical resonance, called a Kondo resonance, that can be correlated with a particular oxidation state—observed in solution-phase experiments—of the molecule.

Again, however, the devil is in the details. In particular, how the single-molecule transistors are made, the way in which molecules are assembled across the junctions, whether the molecules are bound covalently or noncovalently to the electrodes, and what electrode materials are used all play critical roles in either masking or revealing unique molecular electronic properties. Thus, despite early successes, it remains unclear whether single-molecule transistors can emerge as a general spectroscopic tool for guiding the development of molecular electronics.



A universal switch. (Top) Proposed electromechanical switching mechanism in bistable [2]rotaxanes. A positive voltage pulse oxidizes the ground state, resulting in the formation of the metastable state. During the oxidation, the ring (blue) moves from the green to the red site. The ground state is reformed thermally (rate-limiting step) or following a negative voltage pulse. A similar mechanism holds for bistable [2]catenanes (*13*). **(Bottom)** As shown in this Eyring plot, the kinetics of the rate-limiting step depend on the environment, reflecting a slowdown of the switching cycle as the free energy barrier increases from 16 to 21 kcal/mol. Environments: solution; self-assembled monolayer (SAM); polymer; molecular switch tunnel junction (MSTJ).

A second active device is a two-terminal molecular switch tunnel junction (see the first figure, bottom panel). The goal here is to design a molecule that, at a specific voltage, switches from a stable structure (isomer) to another, metastable isomer with a different conductivity and remains in the latter state until either another voltage pulse is applied or thermal fluctuations cause a return to the original isomer. The two states of the molecule correspond to the On and Off states of the switch, and the finite stability of the metastable state leads to a hysteretic current/voltage response that forms the basis of the switch. However, such switching behavior can also arise from the intrinsic device capacitance, from charge storage in defect sites at the molecule/electrode interface, or from electrochemical modification of the electrode materials (*10*). Such artifacts can be ruled out through careful control experiments, but some other, nonmolecular mechanism may nevertheless contribute to the switching response. Thus, the challenge is not just to rule out artifacts, but also to verify that the effect is molecular in origin by establishing a correlation to solution-phase observations.

We have previously reported (*11*) on molecular switch tunnel junctions that contain a monolayer of bistable mechanically interlocked molecules—such as the [2]rotaxane (*12*) shown in the lower panel of the first figure—sandwiched between silicon and metallic electrodes. These devices can be voltage-switched between a stable Off and a metastable On state. For the rotaxane case, we attributed these observations to an electrochemically driven translation (second figure, top panel) of the viologen-containing ring (colored blue) from the tetrathiafulvalene (green) site to the dioxynaphthalene (red) site to form the metastable state. The free energy barrier for relaxation back to the ground state provides an opportunity to correlate the device characteristics with molecular properties in solution.

To establish this correlation, we performed variable temperature electrochemical measurements (*13*) to quantify the metastable-to-ground state relaxation of these molecular switches not only in solution, but also in self-assembled monolayers and in polymer matrices, as well as in the molecular switch tunnel junctions. The free energy barriers to relaxation (see the second figure, bottom panel) of the switches in these four different environments are, respectively, 16, 18, 18, and 21 kcal mol^{-1} at room temperature. Thus, although the corresponding relaxation rates significantly slow down by a factor of 10,000 as the molecules are increasingly confined, the mechanism remains the same: It is universal.

Several other groups have reported theoretical (*14*) and experimental (*15*) studies on similar molecular mechanical systems in various environments. For example, Katz *et al.* have demonstrated a fuel cell in which a rotaxane self-assembled on gold electrode surfaces transports electrons from glucose oxidase to the electrode (*15*). The rotaxane bears a cyclophane that shuttles along the molecular string toward the electrode and back again within about 3 and 12 ms, respectively. Photo-driven relative movements (μs) of the components in a hydrogen-bonded rotaxane have been demonstrated (*16*). Poleschak *et al.* have shown that mechanical movements in bistable, copper-based catenanes and rotaxanes display (*17*) lifetimes of microseconds to hours depending on their structures. The structures of molecular switches can thus govern switching kinetics (*13*). This discovery augurs well for achieving a fundamental goal in the field: chemical control over the physical properties of electronic devices.

Molecular electronics will mature into a powerful technology only if its development is based on sound scientific conclusions that have been tried and tested at every step. Reaching these objectives requires a detailed understanding of the molecule/electrode interface, as well as developing methods for manufacturing reliable devices and ensuring their robustness. Although applications involving single devices already exist (*18*), the next-generation technologies will most likely consist of hybrid devices that combine molecular with existing electronics.

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Silicon Device Scaling to the Sub-10-nm Regime

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In the next decade, advances in complementary metal-oxide semiconductor fabrication will lead to devices with gate lengths (the region in the device that switches the current flow on and off) below 10 nanometers (nm), as compared with current gate lengths in chips that are now about 50 nm. However, conventional scaling will no longer be sufficient to continue device performance by creating smaller transistors. Alternatives that are being pursued include new device geometries such as ultrathin channel structures to control capacitive losses and multiple gates to better control leakage pathways. Improvement in device speed by enhancing the mobility of charge carriers may be obtained with strain engineering and the use of different crystal orientations. Here, we discuss challenges and possible solutions for continued silicon device performance trends down to the sub-10-nm gate regimes.

The steady reduction in the minimum feature size in integrated circuits has helped the microelectronic industry to produce products with spectacular increase in computational capability and integration density at lower cost. Smaller transistors operate faster than larger ones, and for a given chip technology, the cost of a chip decreases with area rather than with the number of transistors.

The exponential scaling trend surely will eventually hit fundamental limits, but the many predictions of a near-term end of device scaling have proven too pessimistic. With the introduction of the production of 90-nm node technology in 2004, the semiconductor industry is entering the "nano" era (*1*). (The "90-nm node" refers to the smallest half-pitch metal lines available in the technology. The actual gate lengths of the devices are about 50 nm.) In the next decade, device gate lengths will be scaled to below 10 nm (*1*). We discuss below the challenges in device scaling and possible solutions in maintaining the performance trend.

MOSFETs: The Building Blocks

The MOSFET, or metal oxide semiconductor field-effect transistor, is a fundamental switching device in very-large-scale integrated (VLSI) circuits. A MOSFET (Fig. 1A) has at least three terminals, which are designated as gate, source, and drain. The gate electrode is separated electrically from the source and the drain by a thin dielectric film, usually silicon dioxide. The source and the drain are doped with impurities that are opposite in polarity to the substrate, which is doped with boron for N-channel transistors and with arsenic or phosphorous for P-channel transistors. This source, substrate, and drain doping effectively produces two back-to-back junction diodes from the source terminal to the drain terminal. When a sufficiently large positive voltage is applied to the gate of an N-channel transistor (which creates an electric field, hence the field effect), the silicon surface is "inverted"—the conduction band is populated and forms a narrow conducting layer between the source and the drain. If there is a voltage difference between the source and the drain, an electric current can flow between them. When the gate voltage is removed or set at zero voltage, the surface region under the gate is depleted with electric carriers and there is no current flow between the source and the drain. We can therefore see that the current flowing through the structure can be regulated by applying voltage to the gate electrode.

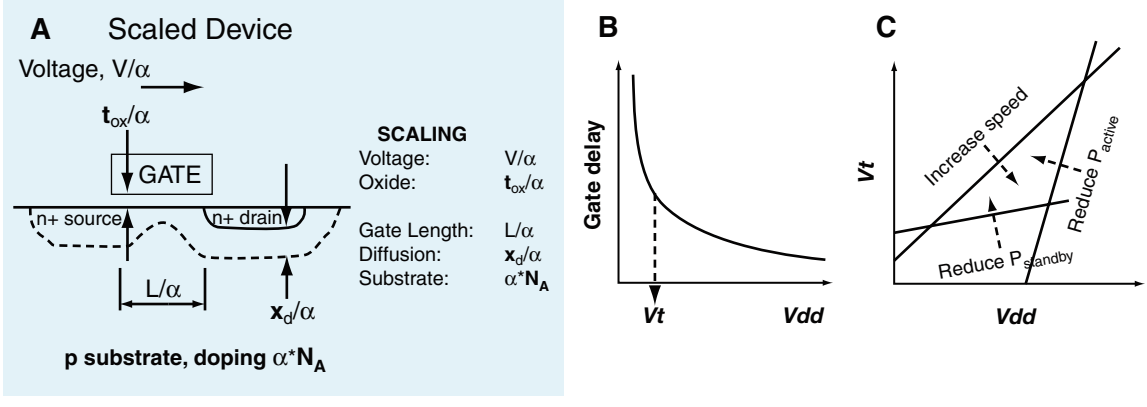

Fig. 1. (**A**) Schematic of MOSFET indicating various relevant device scaling parameters. (**B**) Complementary metal-oxide semiconductor (CMOS) inverter gate delay as a function of power-supply voltage (*Vdd*). Gate delay rapidly increases as *Vdd* approaches the threshold voltage (*Vt*). (**C**) Design space for supply and threshold voltages for optimum performance and power dissipation. Technology scaling diminishes this design space.

A MOSFET can be used either as an electrical switch or as an amplifier. The majority of MOSFETs on an integrated circuit today are used as electrical switches. How fast a MOSFET can be switched on and off is therefore a critical figure of merit to determine the competitiveness of the technology. The two major factors that control the speed of MOSFETs are the channel length from the source to the drain and the speed at which channel charge carriers travel from the source

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to the drain. We will discuss these two factors in more detail below.

Shrinking the Transistor

Shrinking transistors not only packs more devices into a given area but also shortens the distance between source and drain, or the gate length, which can improve the switching speed. The two challenges in decreasing MOSFET size are fabrication and maintaining performance. The backbone of MOSFET fabrication is a process called lithography, which resembles the printing of a photograph by shining light through a negative onto a photosensitive surface. Lithography allows complex patterns to be created through a series of printing and etching steps. The ability to print ever-smaller fine lines is mandatory for continued device scaling. Advanced photolithography techniques have enabled the industry to keep pace with the demand imposed by increasingly smaller structures. However, the smallest feature size is related to the fundamental limit of wavelength used in conventional optical lithography. Alternative technologies capable of writing features far smaller than those produced by conventional optics have also been demonstrated and include the use of extreme ultraviolet (EUV) radiation, x-rays, and electron beams. Recently, the self-assembly process has attracted much attention because of its potential in producing nanoscale patterns. The concern is whether any of these alternatives can be scaled up to meet the throughput, control, and cost requirements for manufacturing.

Given the ability to create smaller device features, to what extent can the gate length be reduced before the MOSFET ceases to function as a switch? The gate terminal can lose the control of channel electric carriers when the source and the drain are brought into proximity without scaling other device parameters. Eventually, the gate terminal cannot turn off the devices, and transistor action can no longer be observed. This phenomenon is the so-called short-channel effect (SCE). According to the scaling theory of Dennard (*2*), the vertical dimensions (gate-oxide thickness, junction depth, and depletion width) must be scaled down with the lateral dimensions such as gate length. This theory guarantees appropriate electrostatic characteristics when a larger device is scaled down to a smaller one (Fig. 1A). The industry has been by and large following this scaling guideline for shrinking MOSFETs to gain higher density and speed without degrading reliability and power.

The accelerated gate-length scaling in the past decade has pushed many vertical device parameters to their fundamental or technological limits. For example, gate oxide now consists of only a few atomic layers; alternative gate materials with a higher dielectric constant are needed. It is also difficult to produce an extremely shallow and abrupt junction without increasing the external resistance. (Junction resistance is inversely proportional to junction depth.)

Ion implantation has been the dominant process for the creation of shallow junctions. A high-temperature annealing process is needed to repair the damage to the single crystal and to activate the dopant atoms. During this step, diffusion and redistribution of dopant atoms occur. A high-temperature, short time scale annealing process is more desirable to produce shallow and abrupt junctions. A near-zero thermal-budget junction technology, such as millisecond flash-lamp and laser annealing, will be required.

Finally, the increase in channel doping needed for SCE control will substantially increase the junction capacitance and leakage. All of these changes degrade device performance. The problem is exacerbated by the ''nonscaling'' factors that arise when the traditional MOSFET design is scaled.

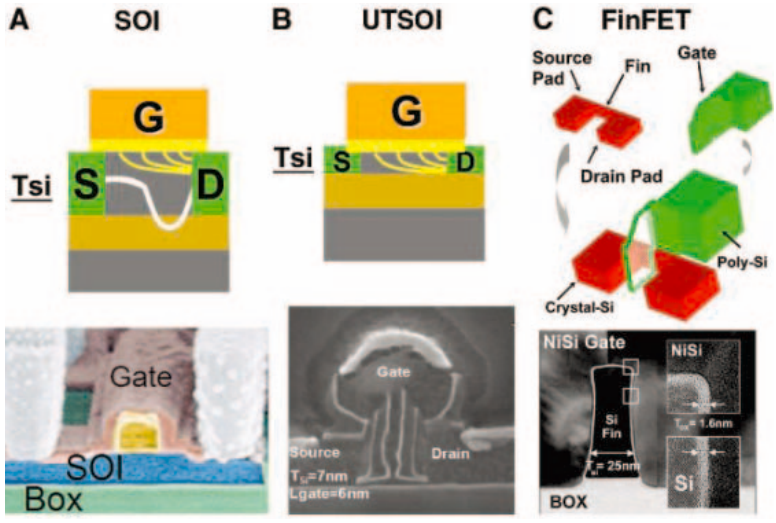

Fig. 2. Device schematics and cross sections for (**A**) SOI MOSFET, (**B**) UTSOI MOSFET, and (**C**) FinFET double-gate MOSFET.

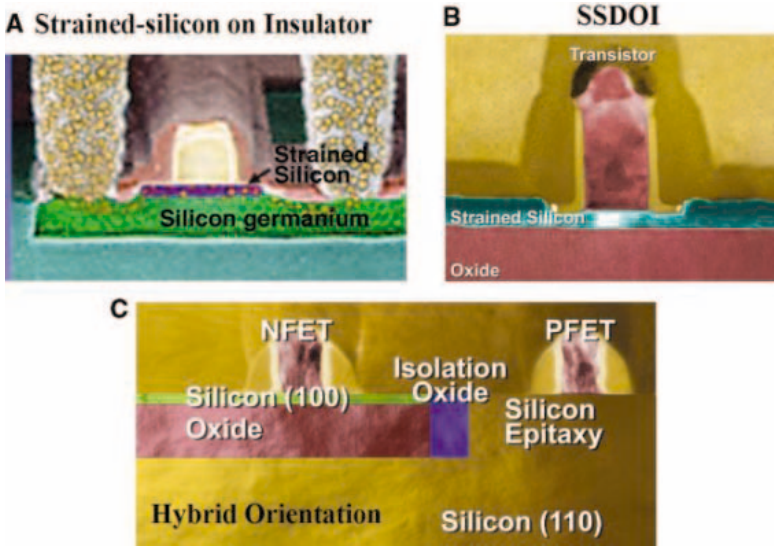

Fig. 3. Device cross sections for (**A**) strained silicon on insulator (SGOI), (**B**) strained silicon directly on insulator (SSDOI), and (**C**) hybrid-orientation technology (HOT).

Nonscaling Factors

As pointed out above, several factors do not scale as we shrink MOSFETs. The subthreshold nonscaling issue is the most fundamental one (*3*). A MOSFET is turned on when a sufficiently high voltage is applied to the gate. The voltage above which the MOSFET is turned on is loosely defined as threshold voltage. The leakage current in the off state depends exponentially on the threshold voltage. Ideally, one would keep the threshold voltage high to minimize the power consumed when the device is off (the stand-by power) and to ensure an appropriate noise margin. The supply voltage is usually reduced in device scaling to keep the active power manageable and to ensure reliability. However, higher device performance will require as much gate overdrive (the excess voltage applied above the threshold) as possible, because higher driving voltages lead to faster switching. Figure 1B shows the circuit delay as a function of supply voltage, *Vdd*. Performance can only be maintained by keeping

threshold voltages low, but the threshold voltage cannot be scaled down much without causing a substantial increase in leakage current in the off state. The proper choice of *Vdd* and threshold voltage for best performance and power tradeoff depends on the application. The design window rapidly diminishes as technology is scaled down (Fig. 1C). In addition, the aggressive reduction in gate dielectric has also caused exponential increase in gate leakage.

Together, these effects have caused the so-called power crisis in the silicon chip industry. Power management has now become the number one issue in any high-performance and low-power application. Currently, there is a consensus that maintaining the device performance trend with conventional device scaling technique is extremely challenging, if possible at all. New device structures and materials will be needed to sustain the current rate of progress in device technology.

Possible Solutions for Device Scaling

Circuit performance can be improved by building conventional MOSFETs on a silicon-on-insulator (SOI) substrate (Fig. 2A). The insulator layer can be created by implanting oxygen ions and then annealing the layer to form an oxide. The addition of an oxide layer below the transistor junction SOI layer can effectively reduce the junction capacitance and leakage current. It also eliminates the so-called "reverse body effect" (*4*) in stacked circuits. As a result, SOI technology offers a faster circuit and consumes less power.

Ultrathin SOI (UTSOI) MOSFET is an attractive option for device scaling, because it can effectively reduce the SCE and eliminate most of the leakage paths (*5*–*7*). For thicker SOI channels, the drain field could easily penetrate to the source side through the channel or buried oxide when the gate length is reduced. However, a thin SOI channel can resolve this problem. Based on this concept, a functional transistor with a gate length of 6 nm was demonstrated (Fig. 2B) by using an ultrathin channel of 4 to 8 nm and aggressive "halo" (*8*) implantation (*6*). This extremely small silicon MOSFET was functional, but its device drive current suffered from channel mobility degradation and high external resistance.

The integration of ultrathin SOI channels into the conventional MOSFET process is quite challenging. The gate and spacer modules must be carefully designed to prevent substantial silicon consumption. The ion implantation process for the source/drain extension should be designed to minimize loss of dopant dose and to avoid complete amorphization of the silicon layer by the ion implantation process. A thin gate spacer coupled with the raised-source-drain process has been shown to be effective in minimizing external resistance without compromising parasitic capacitance. With this new process scheme, improved drive currents were realized (*9*).

Setting and controlling the threshold voltage in devices with such thin SOI layers is also quite challenging. Conventional doping schemes may not be effective as a result of doping fluctuation. One attractive process option is to completely "silicide" the polycrystalline-silicon gate stack by converting the polysilicon to a metal silicide. The gate work function can be adjusted over a substantial range by alloyed silicide and ion implantation to the gate before silicidation (*7*). Although the external resistance can be reduced by process improvement, the mobility degradation in thin SOI channels could be a fundamental issue. The channel mobility is substantially reduced at SOI thicknesses below 10 nm. This decrease may be caused by a "surface roughness"–like scattering mechanism that results from the perturbation of band potential by variations in the SOI layer thickness (*10*).

The ultrathin SOI thickness requirement for SCE control in single-gate FETs can be relaxed by using a more complex "double-gate" FET that offers improved electrostatic gate control of the body. There are many review articles on double-gate devices (*11*–*13*). The symmetric nature in a double-gate FET reduces the depletion width by 50% compared with that of a single-gate structure. In addition, there is no drain-to-body fringing field through the buried oxide (BOX) (Fig. 2A) in a double-gate structure. Numerical simulations indicate that scalability for double-gate FETs improves by a factor of 2.5 to 3 (*5*). Because the double-gate device operates at much lower vertical electric fields, the mobility requirement in double-gate devices can be lower than that of conventional planar MOSFETs (*14*).

Double-gate FETs can be fabricated in planar (*14*, *15*), vertical (*16*), and finlike (*17*–*19*) structures. Of all the double-gate device structures, the FinFET is the simplest to implement. The body of a FinFET device consists of a vertical crystalline silicon wall (Fig. 2C). The gate wraps around both sides of the fin and creates a channel on each side of the fin. In a FinFET, the two channels are perpendicular to the wafer surface and the current direction is parallel to the wafer surface. High current has been demonstrated when the FinFET structure is combined with a raised-source-drain process.

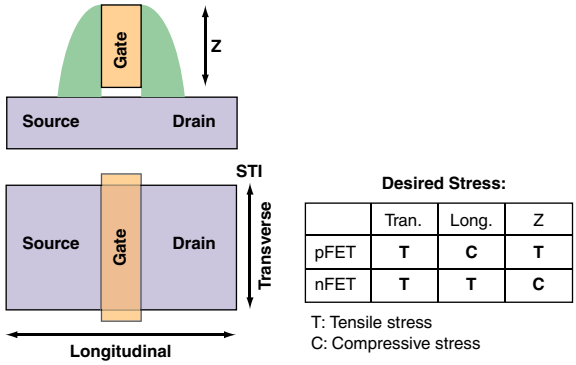

Fig. 4. Possible directions of uniaxial stresses and their effects on N-channel and P-channel MOSFETs.

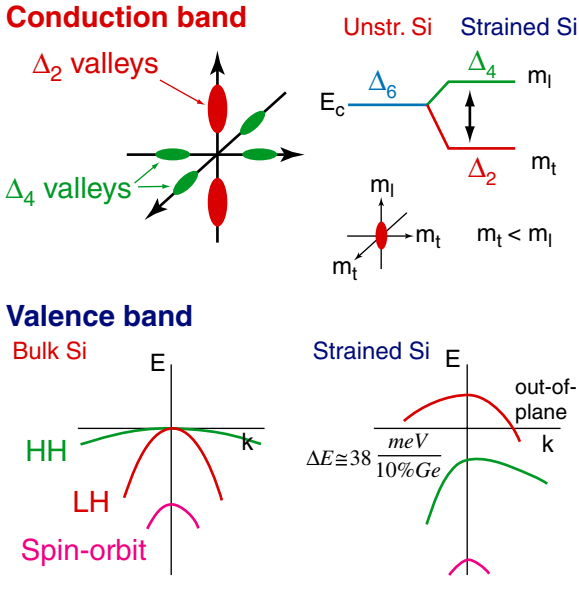

Fig. 5. Biaxial stress effects on the conduction and valence bands of strained silicon.

Enhanced Mobility: Making Carriers Travel Faster

Mobility enhancement is an attractive option, because it can potentially improve device performance beyond any of the benefits from device scaling. The two main approaches being pursued are strain engineering (both process-induced and substrate-induced) and orientation effects (Fig. 3).

Strain engineering. Strain effects induced during the fabrication process can increase the channel mobility. Both tensile and compressive stresses can be introduced in any one of the three dimensions by process techniques (*20*–*27*). The electron and hole mobility respond differently to uniaxial stresses (Fig. 4).

The most effective way to introduce high tensile strain to the channel is to epitaxially grow strained silicon on a relaxed silicon germanium (SiGe) layer. Because of the lattice

mismatch between silicon and SiGe, the lattice of the silicon layer is stretched (strained) in the plane of the interface. This deformation breaks the symmetry of the energy-band structure and results in band splitting (Fig. 5). The reduced interband/intervalley scattering and effective masses result in enhanced carrier transport in the strained-silicon layer that is used as the channel of the MOSFET. Enhanced drive currents of 15 to 25% have been demonstrated on sub-100-nm bulk strained-silicon MOSFETs (*20*). The performance benefit of combining strained silicon with an SOI substrate has also been demonstrated in a 60-nm gate length, N-channel MOSFET with ultrathin thermally mixed strained silicon/SiGe on insulator substrate (*28*). The presence of the SiGe layer in strained-silicon substrate leads to several challenges related to materials and integration, such as a high density of defects in strained silicon on relaxed SiGe induced by the strain relaxation in SiGe and a substantial difference in doping diffusion property in SiGe. (Boron diffusion is retarded, whereas arsenic diffusion is enhanced as compared with the diffusion in silicon.) Such challenges require additional efforts in junction engineering to control SCEs and to set the MOSFET threshold voltages to the desired values. Substantial device self-heating is also observed in strained silicon/SiGe devices because of the lower thermal conductivity in SiGe. Recently, Rim *et al*. (*29*) demonstrated transistors using ultrathin strained silicon directly on insulator (SSDOI) structures that eliminate the SiGe layer before transistor fabrication, thereby providing higher mobility while mitigating the SiGe-induced material and process integration problems.

An SSDOI structure is fabricated by a layer-transfer or ''wafer-bonding'' technique. First, an ultrathin layer of strained silicon is formed epitaxially on a relaxed SiGe layer, and an oxide layer is formed on top. After hydrogen is implanted into the SiGe layer, the wafer is flipped and bonded to a handle substrate. A high-temperature process splits away most of the original wafer and leaves the strained-silicon and SiGe layers on top of the oxide layer. The SiGe is then selectively removed and transistors are fabricated on the remaining ultrathin strained-silicon. A fabricated SSDOI device structure is shown in Fig. 3A. Both electron and hole mobility enhancement have been observed, which indicates that strain is retained after the device-processing steps have been completed (*29*).

Crystal orientation effects. The carrier mobility of inversion layers depends on surface orientations and current flow directions. For P-channel MOSFETs, hole mobility is 2.5 times as high on (110) surface orientation as on a standard wafer with (100) surface orientation (*30*). However, electron mobility is highest on (100) substrates. To fully realize the advantage of the carrier mobility dependence on surface orientation, a new technology has been developed to fabricate complementary metal-oxide semiconductor (CMOS) on hybrid substrates with different crystal orientations, with NFETs on silicon of (100) surface orientation and PFETs on (110) surface orientation (*31*). In this hybrid-orientation technology (HOT), layer-transfer process, block-level trench etch, and epitaxial regrowth were performed before the conventional CMOS device process. A cross section of CMOS on hybrid substrate is shown in Fig. 3C, with NFET on (100) SOI and PFET on (110) silicon epitaxial layer. The hybrid substrate was formed by layer-transfer technique through wafer bonding. First, hydrogen was implanted into an oxidized silicon substrate. The wafer was then flip-bonded to a handle wafer with different surface orientation. A two-phase heat treatment was then carried out to split the hydrogen-implanted wafer and strengthen the bonding. Finally, the top SOI layer was polished and thinned down to the desired thickness. A substantial PFET performance enhancement was demonstrated on 90-nm-node CMOS devices.

Threshold voltage roll-off behavior, junction leakage current, overlap, and junction capacitances are all very similar between (110) and (100) substrates, which indicates similar dopant diffusion characteristics for these orientations. The HOT technology is clearly an attractive option to improve device performance without introducing new material. However, the impact on circuit performance of mixing SOI and bulk devices on the same chip will require more detailed analysis.

Viability: The Crucial Issues

The 2003 version of the International Roadmap for Semiconductors (*1*) projected that, by 2016, sub-10-nm gate-length MOSFETs will be in production with equivalent oxide thicknesses of 5 Å and junction depths below 10 nm. Although functional MOSFETs with sub-10-nm gate lengths have been demonstrated using UTSOI substrate, manufacturability problems of sub-10-nm gate devices remain to be resolved. First, gate stacks with higher dielectric constants and metal gate electrodes are needed to mitigate the gate leakage problem. Second, alternative doping techniques are required to produce shallow and abrupt junction profiles without severely increasing the external resistance. Third, alternative device structures such as UTSOI and double-gate structures will likely be needed for sub-10-nm gate devices.

Additional sources of performance gain are also needed to compensate for any degradation from the subthreshold nonscaling phenomenon. Mobility-enhancement technique is attractive, because it provides performance enhancement in addition to any benefits derived from device scaling alone. Straining the silicon crystal and building N-type and P-type MOSFETs on different crystal orientations are promising methods for mobility enhancement. In fact, some form of strained-silicon techniques are already being used in silicon integrated-circuit manufacturing. Some scaling and mobility-enhancement options can be combined for even higher performance gains. One example that integrates both UTSOI and FinFET devices on the same wafer and that enables hybrid orientation was reported by Doris *et al*. (*32*).

The growing power density and the diminishing process margin of sub-10-nm gate-length MOSFETs cannot be dealt with by process technology alone. Overall system performance gain will require optimization among the technology, circuit, packaging, and architecture levels.

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Human Amygdala Responsivity to Masked Fearful Eye Whites

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The human amygdala has been shown to be activated robustly by fearful facial expressions in neuroimaging studies, even when expressions are presented with backward masking techniques that decrease the temporal availability of facial expression information and mitigate subjective awareness of their presence (*1*). This efficiency in information processing could be consistent with the proposal that the amygdala can respond to crude representations of stimuli (*2*). On the basis of data showing that the eye region of the face is one of the key regions where expression information is extracted (*3*–*6*) and data showing that the amygdala is responsive to the "wide-eyed" expressions of both fear and surprise (*7*, *8*), we hypothesized that the larger size of fearful eye whites (i.e., sclera) would be sufficient to modulate amygdala responsivity.

To test this possibility, we modified standardized fearful and happy face stimuli (*9*) by removing all information from the face but the eye whites (Fig. 1). Because presentation of eye whites alone represents a noncanonical stimulus, we presented these stimuli in a backward masking paradigm to decrease subject's awareness of their presence and, in turn, of their aberrant nature. Grayscale neutral faces were thresholded to create black and white line drawings for use as masks for the eye stimuli (fig. S1C). During functional magnetic resonance imaging, 20 subjects (*10*) viewed neutral face mask presentations, half of which were preceded by fearful eye whites (larger) and half of which were preceded by happy eye whites (smaller).

In separate scans, subjects viewed presentations of "eye blacks" (fig. S1B), inverse, "negative" images of the fearful and happy eye-white stimuli, masked in the same fashion. Because "edge" information was identical in the eye-white and eye-black conditions, the eye-black condition tested whether it was the eye outline that determined amygdala response or the size of the white scleral field. Thus, eye-black stimuli of an identical size, shape, and positioning were presented within-subject to show that the size of the more ecologically valid eye whites is a basic and important stimulus of interest to the amygdala.

Figure 1 shows that signal intensity within the ventral amygdala was greater to fearful than to happy eye whites ($x = -15$, $y = -4$, $z = -19$; $P = 0.0000004$, uncorrected) and also shows the predicted expression by sclera color interaction [$F(1, 19) = 10.69$, $P = 0.004$]. All subjects reported being unaware of the presence of the masked eye stimuli (*11*). No other area of the amygdala was differentially responsive to the fearful versus happy eye-black stimuli ($P > 0.05$). The ventral locus observed here is compelling because in the human, the ventral amygdala comprises the basolateral complex (*12*) where the majority of subcortical and cortical inputs to the amygdaloid system converge (*2*, *7*, *8*). Responsivity here to eye whites, but not to eye blacks, appears to be driven by the size of the white scleral field and not by the outline of the eye, a finding that may be consistent with data showing that the amygdala is more responsive to low than to high spatial frequency information (*13*). Future studies could determine if this is a response to fearful eyes per se or indicates a more general mechanism (e.g., size or intensity). In the interim, this finding augments data showing that the top half of a fearful face is sufficient to produce amygdala response (*4*) by specifically implicating the sclera. Finally, backward masking is shown here to be a useful strategy for examining component processing of faces (*11*).

Facial expressions of emotion are complex configural stimuli. Although there are holistic messages to be discerned (e.g., "that person is afraid of something"), this demonstration offers one example of a simpler rule that a subset of neuronal systems could use to prime additional circuits that will decode more detailed facial information and/or ready response systems for the potential outcomes predicted by this rule (fig. S2).

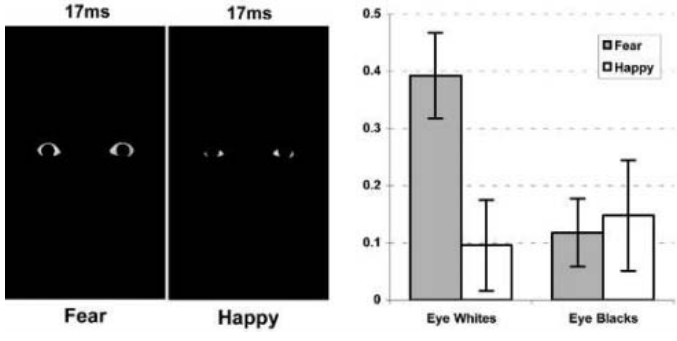

Fig. 1. (**Left**) Examples of the eye-white stimuli. (**Right**) Greater signal increases in the left ventral amygdala occurred to fearful eye whites than to happy eye whites, fearful eye blacks, and happy eye blacks (fig. S1) (*11*). The *y* axis shows the percent signal change from fixation.

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10. We studied healthy, right-handed, male subjects (mean age 21.9 ± 1.34 years) for consistency with our previous study (*1*) and to minimize between-subject signal heterogeneity related to handedness and/or gender differences. We scanned 27 subjects and excluded data from seven for excessive movement (>1.5 mm, 4 subjects), brain or visual abnormalities (2 subjects), or post-scan Beck Depression Inventory scores > 10 (1 subject).
11. Materials and methods are available as supporting material on *Science* Online.
12. We used an imaging protocol focused on the amygdala (*7*) that provides excellent coverage even in ventral and medial regions. The mean signal-to-noise ratio after spatial filtering (full width at half maximum, 6 mm) at the ventral amygdala locus reported here was more than 100 to 1.
13. P. Vuilleumier, J. L. Armony, J. Driver, R. J. Dolan, *Nature Neurosci.* **6**, 624 (2003).
14. We thank N. Kalin, R. Davidson, A. Alexander, R. Cai, H. Urry, and L. Shin. Supported by the National Institute of Mental Health (grant nos. 01866 and 069315) and the Howard Hughes Medical Institute.

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Materials and Methods
Figs. S1 and S2
References and Notes

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United Time-Frequency Spectroscopy for Dynamics and Global Structure

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Ultrashort laser pulses have thus far been used in two distinct modes. In the time domain, the pulses have allowed probing and manipulation of dynamics on a subpicosecond time scale. More recently, phase stabilization has produced optical frequency combs with absolute frequency reference across a broad bandwidth. Here we combine these two applications in a spectroscopic study of rubidium atoms. A wide-bandwidth, phase-stabilized femtosecond laser is used to monitor the real-time dynamic evolution of population transfer. Coherent pulse accumulation and quantum interference effects are observed and well modeled by theory. At the same time, the narrow linewidth of individual comb lines permits a precise and efficient determination of the global energy-level structure, providing a direct connection among the optical, terahertz, and radio-frequency domains. The mechanical action of the optical frequency comb on the atomic sample is explored and controlled, leading to precision spectroscopy with an appreciable reduction in systematic errors.

Ultrashort laser pulses have given a remarkably detailed picture of photophysical dynamics. In studies of alkali atoms (*1*) and diatomics (*2*) in particular, coherent wave packet motion has been observed and even actively controlled. However, the broad bandwidth of these pulses has prevented a simultaneous high-precision measurement of state energies. At the expense of losing any direct observation or control of coherent dynamics, precision spectroscopy enabled by continuous wave (cw) lasers has been one of the most important fields of modern scientific research, providing the experimental underpinning of quantum mechanics and quantum electrodynamics.

This trade-off between the time and frequency domains might seem fundamental, but in fact it results from pulse-to-pulse phase fluctuations in laser operation. The recent introduction of phase-stabilized, wide-bandwidth frequency combs based on mode-locked femtosecond lasers has provided a direct connection between these two domains (*3*, *4*). Many laboratories have constructed frequency combs that establish optical frequency markers directly linked to a microwave or optical standard, covering a variety of spectral intervals. Atomic and molecular structural information can now be probed over a broad spectral range, with vastly improved measurement precision and accuracy enabled by this absolute frequency-based approach (*5*). One of the direct applications is the development of optical atomic clocks (*6*–*8*). To date, however, traditional cw laser-based spectroscopic approaches have been essential to all of these experiments, with frequency combs serving only as reference rulers (*9*).

Here we take advantage of the phase-stable femtosecond pulse train to bridge the fields of high-resolution spectroscopy and ultrafast dynamics. This approach of direct frequency comb spectroscopy (DFCS) uses light from a comb of appropriate structure to directly interrogate a multitude of atomic levels and to study time-dependent quantum coherence. DFCS allows time-resolved studies of coherent and incoherent dynamics: We demonstrate coherent pulse accumulation, quantum interference, and global incoherent optical pumping. At the same time, DFCS exploits massively parallel spectral probing in the frequency domain with a comb bandwidth spanning tens to hundreds of terahertz (*10*), providing a systematic-free connection among various spectroscopic windows of interest. The optical coherence of a phase-stabilized pulse train provides a spectral resolving power approaching that of state-of-the-art cw lasers.

Two-photon DFCS. Our experimental prototype system to study DFCS is a set of two-photon transitions from the ground-state $5S_{1/2}$ to the excited $5D_{3/2}$ and $5D_{5/2}$ states in laser-cooled ^{87}Rb atoms (Fig. 1). The dipole-allowed intermediate states, $5P_{3/2}$ and $5P_{1/2}$, are located ~2 and 17 nm below the energy-degenerate virtual level for the two-photon transition, respectively. The lifetime of the 5P intermediate states is 27 ns, and the lifetime of the 5D states is 240 ns. Also shown (not to scale) is a regularly spaced comb of optical frequencies corresponding to that of the laser. The bandwidth associated with the femtosecond pulse is sufficiently broad that many fine and hyperfine atomic states can be excited by tuning the relevant comb components into resonance. Thus, one laser is used to measure the global energy-level structure while monitoring real-time transition dynamics of the atomic system.

The optical frequency of a particular comb mode can be expressed as $\nu_n = nf_r + f_0$, where f_r is the pulse repetition rate, f_0 is the carrier-envelope offset frequency, and n is an integer on the order of 10^6. Radio-frequency oscillators with ultralow phase noise or ultrastable lasers are used to control the optical comb (*11*). The sum frequency of the light from two comb lines labeled by m and n is given by $\nu_{2\gamma} = (m + n)f_r + 2f_0$. There are several hundred thousand comb pairs (m, n) that yield the same sum frequency and thus could contribute to the transition amplitude when $\nu_{2\gamma}$ is resonant with the two-photon transition ($\delta_{SD} \sim 0$ as shown in Fig. 1). However, it is necessary to consider the intermediate 5P states that provide resonant enhancement. When one of the comb lines is tuned near resonance with one of the 5S-5P transitions ($\delta_{SP} \sim 0$ as shown in Fig. 1), the resonant enhancement causes the corresponding pair to make the dominant contribution to the two-photon transition over all of the other pairs. The dominance is reinforced by destructive quantum interference between comb pairs symmetrically detuned on either side of the P state, resulting in a ~180° phase difference (*12*). For the nonresonant configuration of comb modes detuned $\pm kf_r/2$ ($k \geq 1$, odd integer) away from the P state, with all pairs satisfying the two-photon resonance, the transition amplitudes associated with ($+k$) and ($-k$) modes will again destructively interfere. However, there will be a net nonresonant contribution due mainly to the existence of multiple P states that break the symmetry of the comb distribution. This destructive interference can be made construc-

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tive by flipping the spectral phase about the P state (*12*). Given the two degrees of freedom associated with f_r and f_0, it is always possible to tune a comb to satisfy the two-photon as well as single-photon resonance with any desired intermediate P state. Phase coherence among transition pathways excited by different combinations of comb modes can cause multipath quantum interference effects on the transition probability. The two-photon transition spectrum is obtained by scanning f_r or f_0; their precisely known values, along with (m, n), determine all relevant atomic energy levels in absolute terms.

Traditionally, Doppler-free two-photon spectroscopy has been carried out with two equal-frequency cw laser beams propagating in opposite directions. The two-photon transition rate was resonantly enhanced via available intermediate states using either two different excitation laser frequencies (*13*) or high-velocity atomic beams (*14*). High-resolution two-photon spectroscopy using picosecond pulsed light, hence without any appreciable intermediate-state interaction or absolute frequency reference, has been previously demonstrated (*15*, *16*).

The preceding discussion in the frequency domain, based on an interpretation of the laser spectrum as a set of discrete lines, is complemented by the time-domain multipulse interaction picture (Fig. 1). The pulse-to-pulse optical coherence gives the spectrum its discrete nature, and the time over which optical coherence is maintained determines the width of each line in the comb. The relevant laser parameters for describing the interaction are the interpulse period $\tau = 1/f_r$, the carrier envelope phase evolution between successive pulses $\Delta\phi$, and the pulse duration and its associated area. For times that are short compared to the atomic decoherence time, the femtosecond pulse train drives the atomic coherence in phase such that a multipulse excitation is coherently built up for resonant atomic states. At longer times, however, the coherence in the optical field can no longer be transferred to the atom owing to the finite atomic coherence time. Incoherent processes such as optical pumping then govern the population transfer dynamics. This multipulse interference, combined with a large pulse bandwidth, gives an interesting variation and generalization of the traditional Ramsey technique.

Modeling coherent interactions. The interaction of the femtosecond comb with the atoms was modeled via the Liouville equation for the density matrix of all the atomic states in the laser bandwidth accessible through two-photon absorption, with radiative relaxation included via phenomenological decay terms. The density matrix equations were solved using a perturbative expansion in the field to fourth order. With the approximation of impulsive optical excitation during the pulse, followed by free evolution and decay, an iterative procedure was used to determine the state of the atomic system after any number of pulses (*17*, *18*). The model includes the fine and hyperfine structure of the 5S, 5P, and 5D states, with the Zeeman substructure averaged for linear polarization. A theoretical transition spectrum was constructed with energy-level information provided by the literature, against which we present a comparison with our own measurements. In the simulation, we used calculated oscillator strengths and Clebsch-Gordan coefficients for all involved transition pathways in an effort to accurately predict the relative signal strengths. This general set of Bloch-type equations, evolving from one pulse to the next, leads to a global picture of coherent population accumulation and incoherent optical pumping.

Temporal coherent control is best manifested in the 5D coherent population accumulation and transition linewidth evolution, which through the coherent interaction with the train of femtosecond pulses reach their asymptotic limits imposed by the atomic decoherence. The model results (Fig. 2), under the condition of a small pulse area, illustrate the effect of pulse accumulation on signal strength and spectral linewidth. The on-resonance 5D population increases as the square of the number of pulses until reaching approximately the decoherence time, 480 ns for the 5D states (Fig. 2A). This rapid population increase is accompanied by the narrowing of the resolution linewidth (Fig. 2B), analogous to the spatial resolution and power density scaling versus the number of slits in a multislit experiment. Experimentally we have verified both aspects of this coherent pulse accumulation effect. The effect of the quadratic increase of the excited-state population versus the accumulated number of pulses can be further enhanced with a larger f_r. At long time scales, the global incoherent population transfer has been observed to obey the model prediction.

Experimental method. The experiment was performed with an optical frequency comb (emitted from a 20-fs, 100-MHz repetition rate, mode-locked Ti:sapphire laser) centered at 780 nm with a full width at half maximum bandwidth of ~55 nm (~26 THz). There is ~200 nW of power in the comb lines resonant with the 5S-5P and 5P-5D transitions. The light was typically focused to a beam waist of 130 μm, giving an on-axis intensity of ~0.8 mW/cm^2 in each comb line. For time-resolved studies, the sample was exposed to light by a liquid crystal shutter with a 30-μs response time.

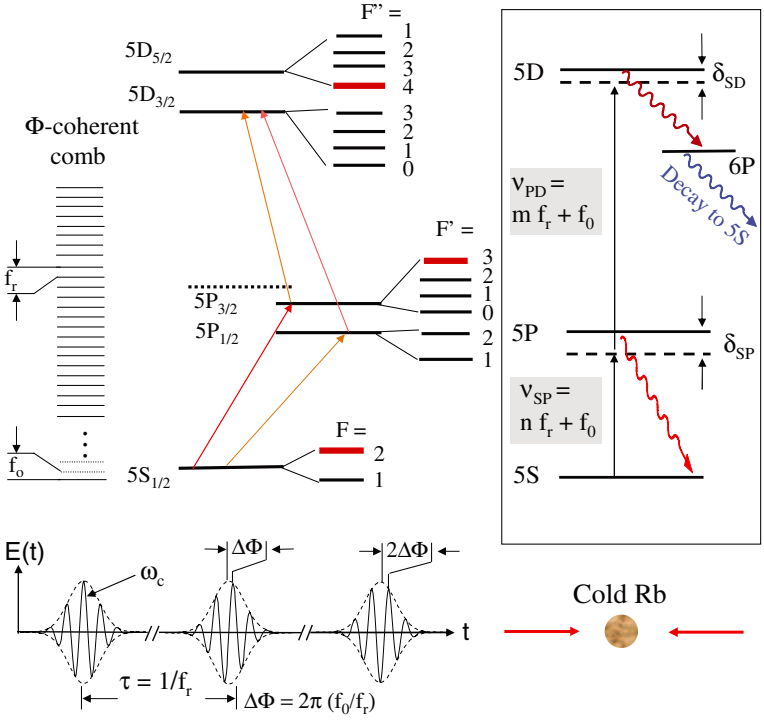

Fig. 1. (**Top**) Schematic of the ^{87}Rb energy levels participating in the 5S-5D two-photon transitions, and the frequency-domain perspective of the atom-light interaction. (**Bottom**) Time-domain picture showing a sequence of mode-locked pulses, with the important interaction parameters τ and $\Delta\phi$. The inset at right shows the relevant "three-level" model used for construction of the Bloch equations to solve for population transfer dynamics. In particular, we show an example of the resonantly enhanced transition with detunings δ_{SP} and δ_{SD} for the pair of comb modes that makes the dominant contribution to the transition probability.

The atomic source was a cloud of ^{87}Rb trapped and cooled in a vapor-cell magneto optical trap (MOT). To minimize magnetic fields during data acquisition, we extinguished the trapping quadrupole magnetic field 2 ms before application of the light from the frequency comb and held the atoms in optical molasses (~20 μK) during this time. The residual magnetic field was compensated by nulling the Zeeman frequency shifts of the two-photon transitions in all three directions using three pairs of bias coils. We extinguished the molasses beams before applying the comb light.

A cw repumping laser controlled the initial populations of the two ground-state hyperfine levels. During the actual probing period, the femtosecond comb itself acts as a repumper, allowing one to maintain a stable population in the initial ground state. The population in the 5D state decays to the 6P state, and the subsequent fluorescent decay at 420 nm from 6P to 5S was detected with a photomultiplier, used in photon counting mode. The counts were then binned on a multichannel analyzer with a typical integration time of 10 μs, and this signal was averaged over hundreds of 20-ms MOT cycles.

Scanning f_r gives a full spectrum. Figure 3 shows the two-photon transition spectrum obtained by scanning f_r for a fixed value of f_0 using linearly polarized light. Also shown is the corresponding theoretical spectrum for the set of parameters used in the experiment. The two insets show signal magnitude on a logarithmic scale to enhance the visibility of the smaller peaks. A total of 39 pathways and 14 transitions have been identified for the $5S_{1/2} \rightarrow 5D_{3/2}$ and $5S_{1/2} \rightarrow 5D_{5/2}$ two-photon resonances. The optical frequency for the two-photon transitions is ~770 THz, an f_r harmonic on the order of 7.7×10^6. Therefore, the two-photon resonance condition is satisfied every time f_r is changed by ~13 Hz, corresponding to a change in the comb frequency by $f_r/2$ for the mode orders around 3.85×10^6. However, the data (Fig. 3) clearly show that the larger, one-photon resonantly enhanced peaks repeat after a change of f_r by ~26 Hz. As mentioned earlier, for the resonantly enhanced peaks, the pair of comb modes that is actually tuned onto the 5S-5P and 5P-5D resonances makes the dominant contribution to the two-photon transition rate.

The peak corresponding to nonresonant excitation of the $5S_{1/2}$ (F = 2) → $5P_{3/2}$ (F = 3) → $5D_{5/2}$ (F = 4) transition, made possible by the collective action of many comb modes, is larger than that theoretically predicted because the comb spectral phase is not flat and the comb spectrum is not symmetric around the P state; thus, the destructive interference mentioned earlier is reduced. Furthermore, as the detuning from the P state becomes greater than a few THz, the effect of phase mismatching between comb pairs over the spatial dimension of the MOT can be non-negligible.

DFCS enables us to measure all of the allowed single- and two-photon transitions within the laser bandwidth by a quick scan of f_r, thus eliminating the need for a broadly tunable and absolutely referenced cw laser. The two resonance peaks observed in the experimental spectrum that are not present in the theory model are due to the $5S_{1/2} \rightarrow 7S_{1/2}$ two-photon transition. For this f_r scan, the initial ground-state population is in F = 2. At longer times (blue curves), all the transitions starting on F = 2 are decreasing in amplitude compared to shorter time scales (red curves) due to ground-state population redistribution and heating. Most of the F = 1 peaks remain unchanged or become larger. Our density-matrix simulation accounts for the time dependence of the shutter response and optical pumping dynamics, but does not include any heating effects. The signal size shown in Fig. 3 has been normalized against the square of the probe power. Not surprisingly, both theory and experiment reveal that the most dominant transition pathway is $5S_{1/2}$ (F = 2) →

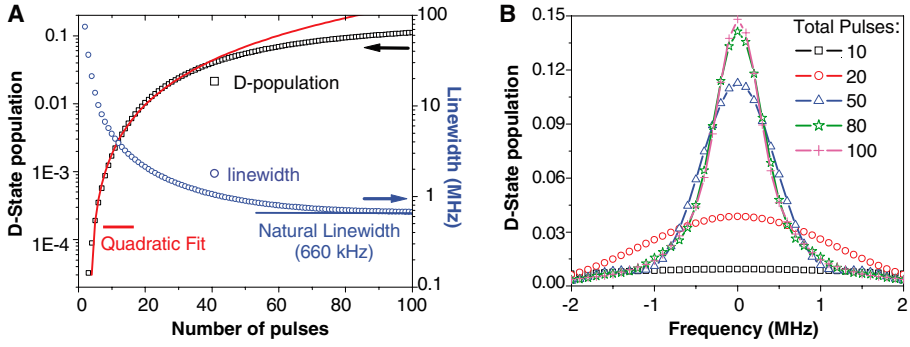

Fig. 2. (**A**) Left (right) axis shows calculated 5D population (linewidth) on resonance for the closed two-photon transition versus total number of accumulated pulses. The time between pulses is ~10 ns, and the system reaches its asymptotic values of signal amplitude and linewidth after ~480 ns. This theory plot illustrates that the DFCS resolution is limited only by the 5D natural linewidth. The quadratic fit to the 5D population at short times shows that the signal scales as the square of the number of pulses until atomic decoherence limits the coherent pulse accumulation. (**B**) The corresponding 5D resonance lineshape versus the number of pulses. In the first 10 pulses, the comb structure is not developed sufficiently to offer appreciable signal or resolution.

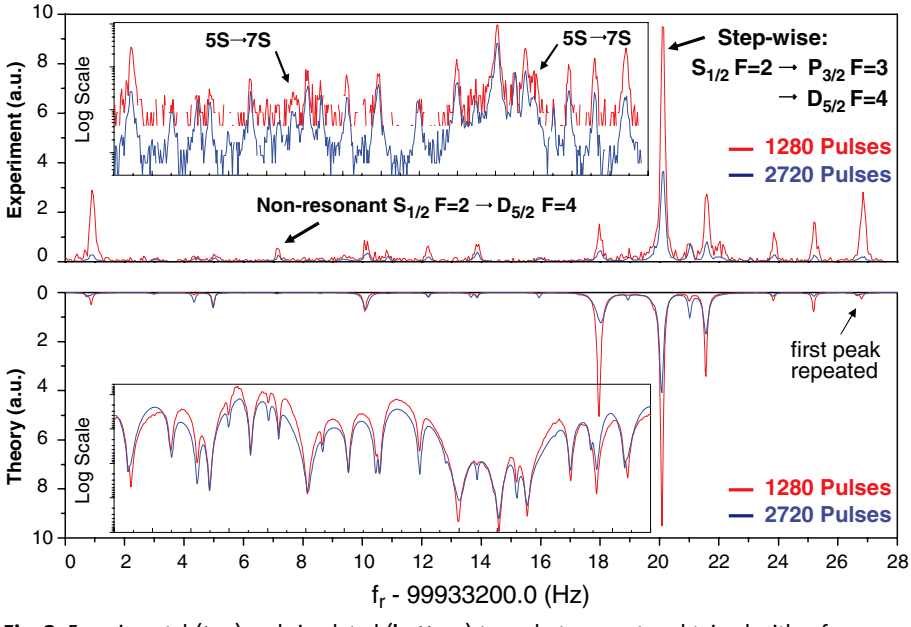

Fig. 3. Experimental (**top**) and simulated (**bottom**) two-photon spectra obtained with a frequency scan of f_r. Shifting f_r by 26 Hz shifts the comb spectrum by 100 MHz, or f_r, approximately repeating the original comb structure. The changes in relative peak sizes from the spectrum obtained after 1280 pulses (in red) to that after 2720 pulses (in blue) illustrate population transfer dynamics by optical pumping as well as heating effects. All 28 possible 5S → 5D two-photon transitions are observed, including resonantly enhanced and nonresonant cases. Two resonances in the data due to the 5S → 7S transition are not included in our model.

$5P_{3/2}$ (F = 3) → $5D_{5/2}$ (F = 4), which is the only closed transition.

The relative size of the features in Fig. 3 after any fixed number of pulses reflects intermediate-state resonant enhancement as well as population transfer. Thus, the spectrum contains all of the fine and hyperfine structure pertinent to the 5D states, while spectroscopy of the 5P states is performed via their resonant enhancement of the two-photon transition as a function of δ_{SP}. We defer further discussion of the P-state spectroscopy, however, until we have explored two important sources of possible systematic error: the mechanical action of the light on the atoms and AC Stark shifts.

Mechanical action of the probe. Although the sub-Doppler temperature established by polarization-gradient cooling provides an ideal initial condition for spectroscopy, it cannot be expected to survive the momentum transferred by the light from the comb. At best, the mean-square momentum will increase, leading to Doppler broadening. Worse, the momentum acquired may lead to systematic shifts in the resonance line positions. Thus, we seek to understand momentum transfer from the comb and to mitigate its effects insofar as possible.

We expect that the momentum transfer is dominated by the interaction of the comb mode closest to resonance with the 5S-5P transition, given that the radiative decay rate of the 5P-5S transition is an order of magnitude greater than that of 5D-5P, and the population largely resides in the 5S state. For ease of modeling, we used a single-beam (traveling wave) configuration and chose a comb structure such that the closed $5S_{1/2}$ (F = 2) → $5P_{3/2}$ (F = 3) → $5D_{5/2}$ (F = 4) transition is dominant. The temporal signal evolution is shown in Fig. 4 for the initial detunings of $\delta_{SP} = 0$ (○), $\delta_{SP} = 1$ MHz (▽), and $\delta_{SP} = 2$ MHz (◇). In the fully resonant case (○), the delayed signal peak at 30 μs reflects the switching time of our liquid crystal shutter. The subsequent decay arises as the accumulated momentum transfer gradually blue-shifts the atoms out of two-photon resonance. Although the 5S-5P transition controls the mechanical action, the two-photon resonance condition plays a more decisive role in the observed signal decay due to the 10-times-narrower D-state linewidth. When $\delta_{SP} = 1$ MHz (▽), the atoms, initially with $\delta_{SD} = 2$ MHz, will only reach the peak of the two-photon resonance after they have reached the velocity at which the Doppler shift compensates the initial detuning. Beyond this velocity, the signal contribution decreases. Similarly, when $\delta_{SP} = 2$ MHz (◇), the signal peaks still later.

All three experimental results agree well with our theory (solid lines in Fig. 4) based on a simple two-level dissipative light force model. The peak position is determined by the scattering rate, photon recoil, and the initial detunings. The width and shape of the peak are associated with the stochastic nature of radiation pressure, the intensity variation over the radial beam profile, and the finite laser linewidth. It is evident that the force exerted on an atom by the optical comb is well modeled by the radiation pressure due to a single comb mode tuned close to the 5S-5P resonance.

As a first step in mitigating the effect of light-induced momentum transfer, we used intensity-balanced counterpropagating beams. Even though the pulses do not overlap temporally inside the atomic cloud, they do interact with the same group of atoms within the atomic coherence time, leading to a

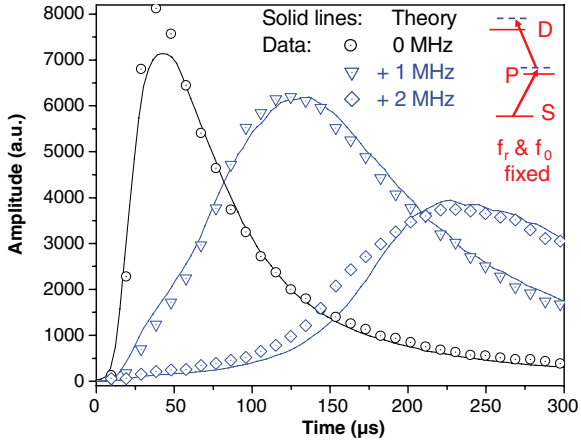

Fig. 4. Momentum transferred by the optical frequency comb (in a single probe beam configuration) to the cold Rb atoms, observed via the time-dependent fluorescence signal from the 5D states. The optical comb has fixed values of f_r and f_0 to achieve the desired detunings (with respect to atoms initially at rest) of $\delta_{SP} = \delta_{SD} = 0$ (black circles); $\delta_{SP} = 1$ MHz, $\delta_{SD} = 2$ MHz (blue triangles); and $\delta_{SP} = 2$ MHz, $\delta_{SD} = 4$ MHz (blue squares), respectively, for the dominant pair of comb modes.

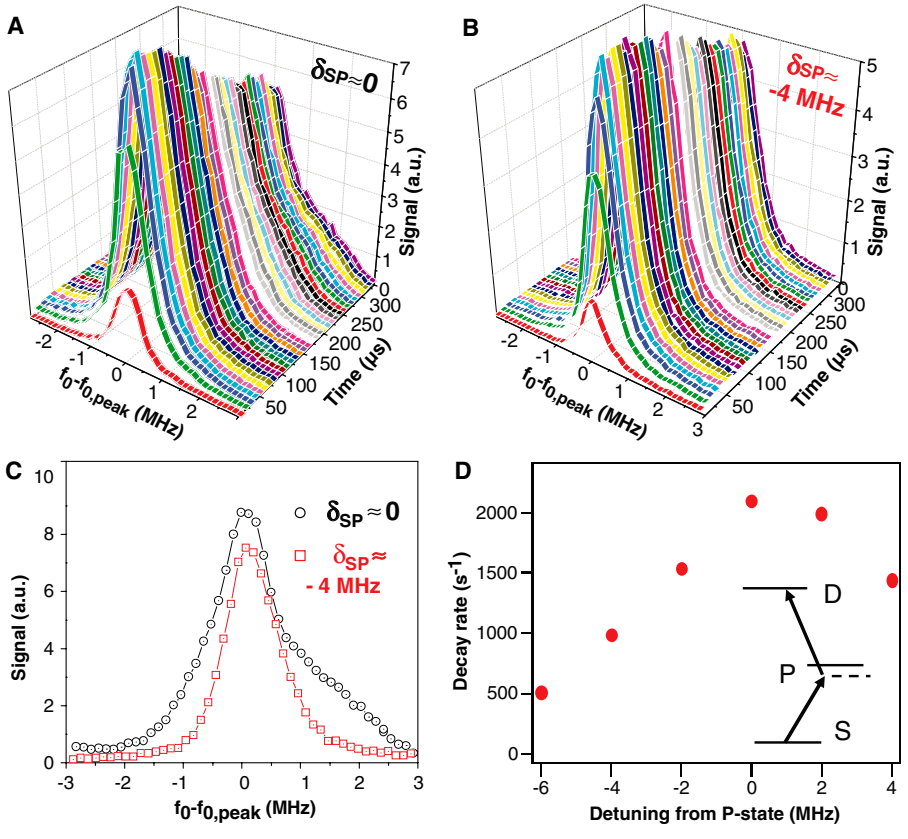

Fig. 5. Time evolution of the 5D fluorescence signal lineshape showing the mechanical action of the optical comb for the case of two balanced counterpropagating probe beams. f_r is fixed while f_0 is scanned over the 5D resonance profile. At the f_0 value corresponding to the expected line center, there are two cases of P-state detuning, (**A**) $\delta_{SP} \approx 0$ and (**B**) $\delta_{SP} \approx -4$ MHz. (**C**) A detailed comparison of the lineshape under the two detuning conditions after an interaction time of 300 μs. (**D**) The decay rate of the peak signal is reduced when the comb mode is tuned below, rather than on or above, 5S-5P resonance; thus, appropriately engineered comb modes can mechanically confine atoms for longer interrogation times.

signal enhancement by a factor of 4 compared to the single-beam case. Again we studied the momentum transfer associated with the $5S_{1/2}$ (F = 2) → $5P_{3/2}$ (F = 3) → $5D_{5/2}$ (F = 4) transition, fixing f_r and scanning f_0. Figure 5, A and B, show time evolutions of the detected signal as f_0 is swept, for two different detuning conditions: $\delta_{SP} = 0$ in Fig. 5A and $\delta_{SP} = -4$ MHz in Fig. 5B, when f_0 is tuned to the two-photon resonance peak. As f_0 is scanned to recover the resonance lineshape, the value of δ_{SP} changes. It is clear that the directed momentum transfer seen in Fig. 4 is greatly suppressed, and the heating is more evident. For the case of $\delta_{SP} \approx 0$, the lineshape profile centered at 300 μs (Fig. 5C, black circles) shows a marked asymmetry. This feature is easily understood, because the comb lines are tuned blue relative to both the 5S-5P and 5S-5D resonances, although the detuning of 5S-5P is less dramatic because its linewidth is an order of magnitude larger. Thus, Doppler heating accompanies the probe of the blue side of the two-photon resonance.

For the case of $\delta_{SP} \approx -4$ MHz, the intermediate-state detuning is always red as the two-photon resonance is probed. The red-detuned comb mode helps to maintain a symmetric absorption lineshape even after 300 μs, as confirmed by the corresponding profile (Fig. 5C, red squares). Thus, a judicious choice of comb structure can help to mitigate the heating of the sample caused by the probing beam. From Fig. 5, A and B, we also observe quite different signal decay rates versus observation time. A reduced signal decay rate attributed to less heating in the red-detuned case compared to the blue-detuned case is reflected in the asymmetric curve around $\delta_{SP} = 0$ (Fig. 5D).

AC Stark shift. Another systematic source of error is the light-induced AC-Stark shift on various atomic states to be measured. To assess this effect in the presence of many comb modes, we again took advantage of the flexibility in control of f_r and f_0 to vary δ_{SP} while keeping δ_{SD} nearly zero for the closed transition. In this near-resonance stepwise transition case, a nonvanishing value of δ_{SP} causes a shift in the measured two-photon transition frequency (*19*). To clearly distinguish the AC-Stark shift from mechanical actions, we gradually increased the power of the pulses as the laser shutter opened. For both $\delta_{SP} = +4$ MHz (△) and $\delta_{SP} = -4$ MHz (□) cases, the AC Stark shifts are present as soon as the laser is turned on and the transition frequency shift follows the time evolution of the peak power of the pulse train (Fig. 6A). When $\delta_{SP} = 0$ (○), the measured AC Stark shift is close to zero when the shutter just opens. The frequency shift measured at later times is attributed to the accumulated photon momentum transfer, which is reduced in the detuned cases. Again, the solid lines represent theoretical results obtained from a simple model of the AC-Stark shifts and mechanical action. The asymmetry in frequency shift between the red and blue detunings is caused by the presence of other 5P hyperfine states that also perturb the 5S-5D transition.

Although the laser spectrum spans roughly 26 THz, the obtained spectroscopic resolution approaches the atomic natural linewidth. This level of resolution is a result of the use of ultracold atoms and careful control of the phase-stabilized comb parameters, stray magnetic fields, light-induced shifts, and photon-momentum transfer. We typically measured two-photon linewidths on the order of 1 MHz, which is consistent with the convolution of the natural linewidth of 660 kHz and the laser technical linewidth of 300 kHz. The measured transition linewidth is slightly smaller for red detuning ($\delta_{SP} = -4$ MHz) than blue detuning (at the same $|\delta_{SP}|$ value) with the same probe power, again showing the benign effect of mechanical action by the red-detuned comb mode.

Absolute frequency measurement. With the understanding of systematic effects, we have analyzed spectra similar to the ones shown in Figs. 3 and 6A to construct a table of absolute transition frequencies from 5S to 5P and to 5D (Table 1). Some representative transition frequencies are determined directly from the comb structure and are given in the table, along with comparisons to available published values (*20*–*22*). Without any previous information of the 7S energy levels, we have also determined their absolute transition frequencies (*23*) by scanning the resonances for two sufficiently different values of f_r to determine the corresponding comb mode numbers. A single optical comb thus provides atomic structural information in the optical, terahertz, and radio-frequency spectral domains. The measurement accuracy is currently a few kHz to a few tens of kHz for the D states and on the order of 100 kHz for the P states, comparable to the highest resolution measurements made with cw lasers. To determine the absolute frequencies of the 5S-5P transition, we have scanned the P state directly, using a set of f_r and f_0 pairs that have a range of detunings from the P state, and are all two-photon resonant. Retrieving the actual P-state lineshape requires normalization based on our density matrix model to remove the optical pumping caused by varying detunings from other P states.

Implications and applications of DFCS. The resolution of DFCS can be improved by locking the femtosecond laser to a cavity that has been used to reduce the line-

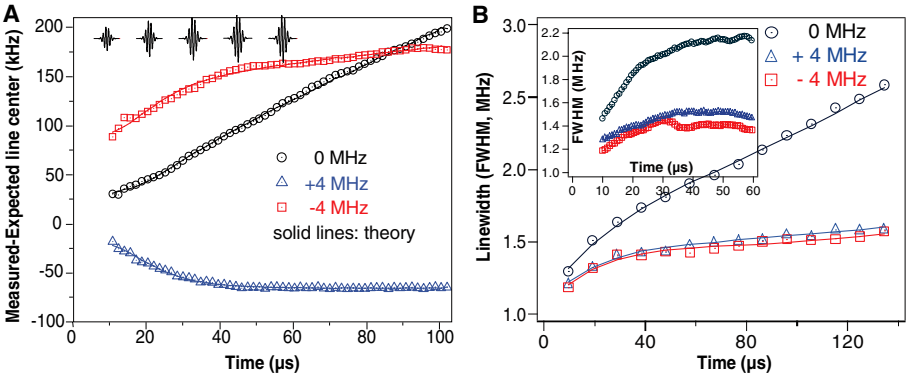

Fig. 6. Measurement of (**A**) the line center frequency and (**B**) linewidth for the closed two-photon transition, revealing frequency shifts from both the AC-Stark effect and mechanical action. Extrapolating to zero interrogation time and zero power yields the corrected atomic transition frequencies (A) and the natural transition linewidths (B).

Table 1. Rb level structure from direct frequency comb spectroscopy. All values are obtained by extrapolating the line-center to zero probing time and power.

Measured transition (from $5S_{1/2}$, F = 2)	Measured frequency (kHz)	Literature value (kHz)*
$5D_{5/2}$ F = 2	770,569,184,527.9 (49.3)	770,569,184,510.4 (16.0)
$5D_{5/2}$ F = 3	770,569,161,560.5 (11.1)	770,569,161,555.6 (16.0)
$5D_{5/2}$ F = 4	770,569,132,748.8 (16.8)	770,569,132,732.6 (16.0)
$5D_{3/2}$ F = 3	770,480,275,633.7 (12.7)	770,480,275,607.6 (10.0)
$5D_{3/2}$ F = 2	770,480,231,393.9 (38.1)	770,480,231,385.2 (10.0)
$5P_{3/2}$ F = 3	384,228,115,309.0 (63.0)	384,228,115,203.3 (7.1)
$5P_{1/2}$ F = 2	377,105,206,938.7 (179.0)	377,105,206,705.0 (400.0)

*From (*20*–*22*).

width to below 100 Hz (*24*). Similarly, a larger signal can be obtained by using a laser with a higher repetition rate; for example, a 1-GHz laser with the same average power and spectral width could increase the signal up to a 100-fold (*25*). One practical consequence of these results is a method to control both degrees of freedom of the femtosecond comb directly by an optical transition in cold atoms. Another interesting application of the demonstrated pulse accumulation effect is laser cooling of atoms that require coherent ultraviolet light not easily accessible by conventional laser sources (*26*). For general coherent control experiments, pulse accumulation (when enabled by long coherence times) can complement spectral amplitude and phase manipulations, leading to improved efficiency in population control with the added spectral resolution due to multipulse interference. The precise and phase-coherent pulse accumulation may prove particularly useful in efficiently populating atomic Rydberg states for quantum information processing. Although the current experiment involves two-photon transitions, the advantages of DFCS should apply equally to single-photon and multiphoton excitations. Multiple ultrafast lasers with optical spectra independently tailored for different spectroscopic features could be phase coherently stitched together (*27*, *28*) to further extend the utility of this approach.

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REPORTS

Building Programmable Jigsaw Puzzles with RNA

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One challenge in supramolecular chemistry is the design of versatile, self-assembling building blocks to attain total control of arrangement of matter at a molecular level. We have achieved reliable prediction and design of the three-dimensional structure of artificial RNA building blocks to generate molecular jigsaw puzzle units called tectosquares. They can be programmed with control over their geometry, topology, directionality, and addressability to algorithmically self-assemble into a variety of complex nanoscopic fabrics with predefined periodic and aperiodic patterns and finite dimensions. This work emphasizes the modular and hierarchical characteristics of RNA by showing that small RNA structural motifs can code the precise topology of large molecular architectures. It demonstrates that fully addressable materials based on RNA can be synthesized and provides insights into self-assembly processes involving large populations of RNA molecules.

DNA has been extensively used to generate artificial geometrical objects like polyhedra (*1–3*), various self-assembling two-dimensional (2D) nanostructures (*1*, *4–6*), and DNA nanomechanical devices (*7–9*). Seeman, Winfree, and collaborators (*1*, *4*, *5*) have shown that DNA tiles based on various "crossover" DNA motifs could assemble in a predictable manner into periodic and aperiodic patterned 2D arrays. These DNA arrays are still made of a limited number of distinct molecular tiles and display rather simple patterning with no finite dimensions. However, their work suggests that versatile programmable molecular systems capable of algorithmic assembly into an infinite variety of 2D or three-dimensional (3D) supra-architectures with increasing pattern complexity, shape, molecular diversity, and size could potentially be generated with nucleic acids (*10*).

Although more chemically labile than DNA, natural RNAs offer a richer treasure trove of rigid structural motifs (*11–14*) that can be potential modules for supramolecular engineering (*15–20*). RNA tectonics (*15*) refers to the modular character of RNA, which can be decomposed and reassembled to create new RNA nanoscopic architectures. With the idea in mind to generate addressable materials with increasing patterns of complexity and molecular diversity, we have used a sequential stepwise assembly strategy to construct programmable building blocks with RNA tectonics. These molecules behave as "smart" RNA pieces, which could ultimately self-assemble in a predictable manner into any possible 2D architecture with full control over size, shape, and pattern geometry. Thus, the final position of each molecule can eventually be known and, therefore, be addressable, within a molecular jigsaw puzzle of finite size.

At a molecular level, "square-shaped" RNA supramolecules with sticky, interacting tails can potentially be programmed to assemble into many different planar networks of predefined geometries. We chose

two small RNA structural motifs present in the ribosome crystallographic structures (*12*–*14*) to guide our design of a self-assembling square made of four similar but nonidentical subunits, called tectoRNAs (Fig. 1, A and B) (*21*). Each tectoRNA contains two interacting hairpin loops (*19*, *22*) covalently joined by a small structural motif of 11 nucleotides, called the right angle (RA) motif, that specifies 90° angle corners between adjacent helices within the context of the ribosome (*12*, *13*). To avoid homomultimers, the formation of a closed, circular tetramer is directed by four distinct, specific noncovalent loop-loop interactions, called kissing loop (KL) complexes (*22*), which are expected to adopt collinear extended helical structures according to the crystallographic structures of the ribosome (*12*) and the dimerization initiation site of human immunodeficiency virus (HIV) RNA (*22*). A tectosquare 3D model resembles a square when viewed from the top. Nevertheless, it is not flat, because its extended helical sides adopt a log cabin–like conformation at the level of RA corners (Fig. 1C; fig. S1). Rather than being a perfect four-fold pseudosymmetrical object (C_4), the tetramer has two-fold pseudosymmetry (C_2). Small (ST) and large (LT) tectosquares, with 10-nm and 13-nm side lengths, can be constructed from tectoRNAs with hairpin stems of 9 and 15 base pairs (bp), respectively.

Tectosquares can further self-assemble through specific sticky tail connectors (Fig. 1). The tails of the tectoRNAs can be designed to have a wide variety of sequences. Their precise positioning and orientation are inferred from the RA motif geometry. The 3′ tail, stacked in continuity to the 3′ stem-loop, is expected to be structurally more constrained and directional than the 5′ tail. By swapping the RA motif, the orientation of the 3′ tail can be modified by 90° without changing the overall positioning of the stem-loop arms (Fig. 1D). Moreover, small variations in the tail length can change the overall length of tail connectors by one-half of a helical turn, which positions two adjacent tectosquares in either a cis or trans configuration (Fig. 1E). About 88.5 million distinct tectosquares can be built with a limited set of 12 tail connectors with two different tail orientations and sizes (*23*).

We constructed two sets of tectoRNAs for building ST and LT tectosquares (*21*). Each tectoRNA sequence was optimized to favor folding into a unique, stable secondary structure (*17*, *24*). After being synthesized by run-off transcription, tectosquare modular assembly was monitored by native polyacrylamide gel electrophoresis (PAGE). Magnesium is absolutely required for assembly. At 0.2 mM $Mg(OAc)_2$, an equimolar mixture of each tectoRNA set forms 60 to 90% of a circular supramolecular species that migrates slower than monomers and linear tetramers lacking one of the four KL motifs (Fig. 2A). Remarkably, both tectosquares can be purified out of native PAGE

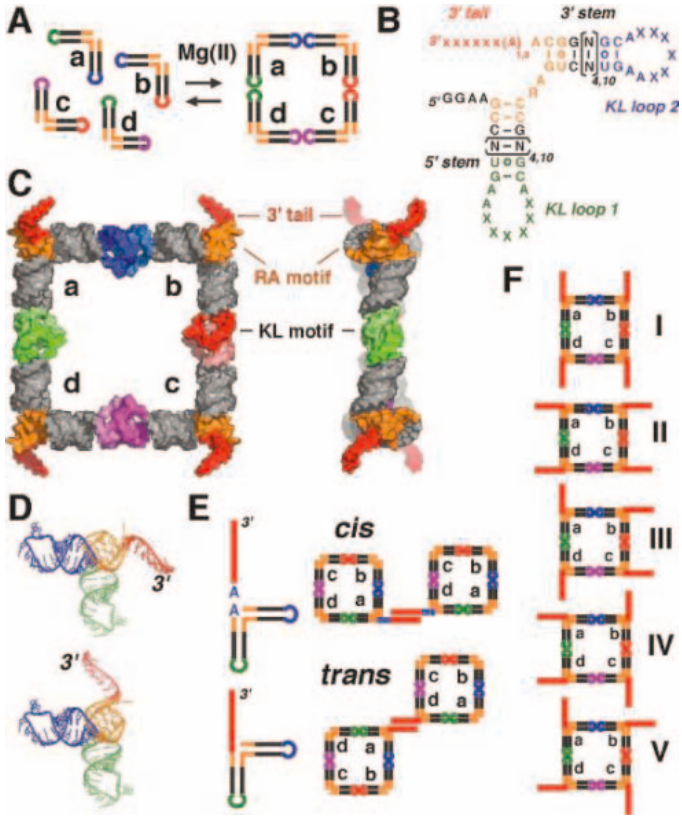

Fig. 1. Tectosquare structure and assembly principles. (**A**) Assembly scheme. (**B**) TectoRNA 2D diagram: LT tectoRNAs have stems 6 bp longer than ST tectoRNAs. N, nt positions involved in stems; x and X, nt from the 3′ tail (in red) and KL loops (in green and blue) involved in Watson-Crick bp for tail-connectors or KL motif formation, respectively. RA motif consensus sequence is in orange. (**C**) Tectosquare 3D model (LT). Front and side views are shown. KL loops form four sequence-specific KL motifs (in blue, red, magenta, and green) that adopt collinear topologies (see also fig. S1). (**D**) Change of 3′ tail directionality upon RA motif swapping. (**E**) Tectosquare cis and trans assembly configurations. (**F**) The five types of tectosquares used in this study.

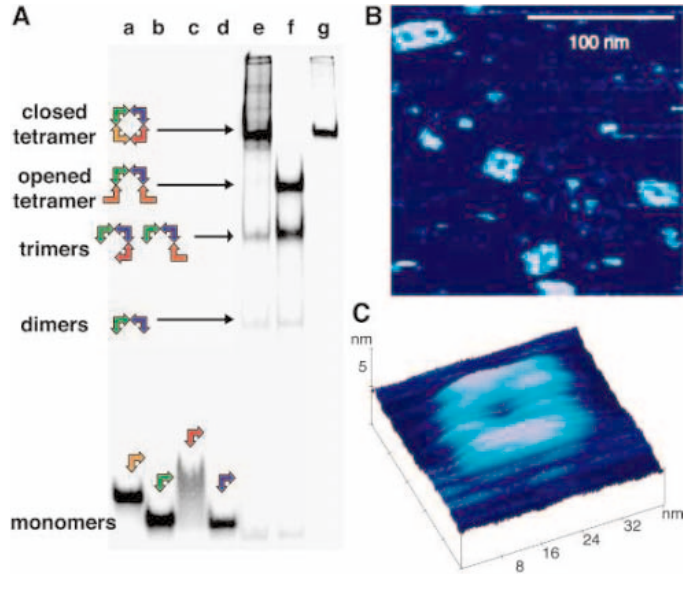

Fig. 2. RNA tectosquares are stable and stiff supramolecular assemblies. (**A**) Nondenaturing PAGE at 0.2 mM $Mg(OAc)_2$ of various combinations of LT tectoRNAs. Lanes a, b, c, and d: tectoRNAs, a, b, c, and d, respectively, at a final concentration of 20 nM. Lane e: equimolar mixture of a, b, c, and d (20 nM each). Lane f: equimolar mixture of tectoRNAs a, b, e, and f (20 nM each). Units e and f assemble with a and b, respectively, but prevent the formation of a circular complex. Lane g: tectosquare after nondenaturing PAGE gel purification and elution at 4°C in the presence of 15 mM $Mg(OAc)_2$. Tectosquares can be kept at 4°C for several days without showing any sign of dissociation or degradation. (**B** and **C**) AFM visualization of LT tectosquare in solution on mica surface (B) or in air after precipitation of the RNA on mica coated with poly-L-lysine (C).

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gels without dissociating (Fig. 2). We investigated further tectosquare stability by temperature-gradient gel electrophoresis (fig. S2), a method for separating different assemblies on the basis of temperature-dependent conformational change (*25*). In 15 mM Mg^{2+}, tectosquares are stable up to 56°C because of the presence of the two structural motifs encoded within their sequence. With equilibrium constants of dissociation (K_d) ranging from 1 to 20 nM at 0.2 mM Mg^{2+}, KL motifs are more stable than RNA duplexes of identical sequences (*19*, *26*). Moreover, tectosquares that contain the RA motif in each of their units are 4.2°C (ST) to 5.8°C (LT) more stable than those without any RA motif (fig. S2). Accordingly, the thermal stability of a tectosquare increases with an increasing number of RA motifs present within its assembly.

The overall topology of an LT was investigated by atomic force microscopy (AFM) (*21*, *27*) in air or in 15 mM $Mg(OAc)_2$ solution after deposition of the RNA sample solution on a mica surface. The predicted and observed tectosquare architectures (Fig. 2, B and C) are in remarkable agreement with each other and unambiguously establish the RA and KL motifs as autonomous folding modules outside their natural context. The LT folds into a square shape with stiff, straight sides of 13 ± 3 nm and corners between 70° and 110°. The central cavity has an overall size of 8 ± 3 nm, and the width of the RNA helical region is estimated to be 3 ± 1 nm at half height, the expected width for a double-stranded RNA helix. The seldom-observed rhombus shape for LT is indicative of a tilted rather than flat tetramer that could be deformed when forced to lie on the mica surface.

The stable, rigid characteristics of tectosquares are particularly attractive for building programmable planar supra-architectures. To demonstrate the predicted geometrical properties of tectosquares described above (Fig. 1), we designed 12 specific 3′ tail connectors of 6 bp and used them to program the assembly of nine different sets of tectosquares into distinctive periodic fabrics (Fig. 3A). The connectors have similar free energies of formation, chosen to be less stable by at least two orders of magnitude than those of KL complexes. At 15 mM Mg^{2+}, two tectosquares joined by two parallel tail connectors disassemble around 30°C; that is, 25°C below tectosquare melting temperature. A simple monitoring of the RNA annealing temperature can thus be used to hierarchically control the assembly process by uncoupling tectosquare association from fabric formation. Forty-nine tectoRNAs with different sizes, tail sequences, tail lengths, and orientations were synthesized and combined to separately construct a total of 22 tectosquares that were then appropriately mixed to generate the nine tectosquare patterns. Pattern formation was performed at 15 mM Mg^{2+}, on the mica surface, by slow cooling from 50° to 4°C and was monitored by AFM under

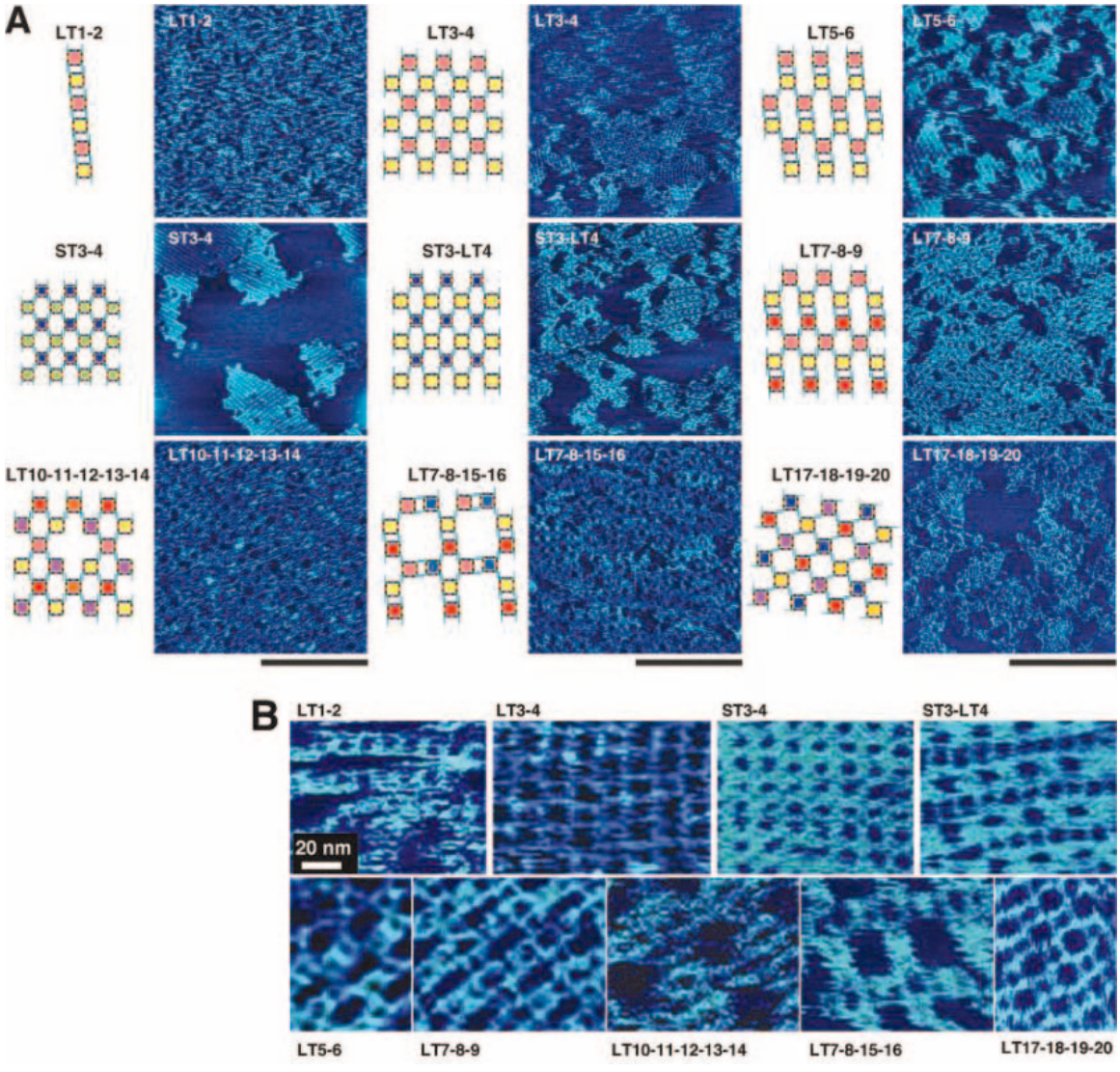

Fig. 3. Diagram and AFM images of tectosquare nanopatterns generated from 22 tectosquares. (**A**) One micrometer square scale AFM images obtained in solution for: LT1-2, ladder pattern; LT3-4, fish net pattern; LT5-6, diamond pattern; ST3-4, striped velvet pattern; ST3-LT4, basket weave pattern; LT7-8-9, lace pattern; LT10-11-12-13-14, polka dot pattern; LT7-8-15-16, tartan pattern; LT17-18-19-20, cross pattern. Scale bars, 500 nm. (**B**) Magnification of patterns in (A). Scale bar, 20 nm. (See also fig. S3.)

aqueous conditions, similar to those used for native PAGE.

We first designed two distinct pairs of large tectosquares, LT1-2 and LT3-4, characterized by the same four tail-connector sequences oriented parallel to "a-d" and "b-c" (LT type I, Fig. 1F), but with tails of 12 and 10 nucleotides, respectively. LT1-2, with connectors of length equivalent to 14 bp, adopts a cis configuration (Fig. 1E) that leads to ladders of 10 to 20 LT (Fig. 3 and fig. S3). By contrast, LT3-4, with connectors of length equivalent to 10 bp, assembles in a trans configuration and forms fish net–like 2D lattices involving up to 100 LTs. In the ladder and 2D lattice arrangements, each LT contacts two and four other LTs, respectively (Fig. 3 and fig. S3). The next set, LT5-6, presents a combination of two of the previous pairs of long and short connectors. This molecular arrangement leads to the formation of stable tectosquare dimers assembling into diamond-like arrays that are as large as LT3-4 arrays but with meshes twice as big (14 ± 2 nm by 27 ± 3 nm) (Fig. 3). STs efficiently assemble into predetermined architectures as well. The ST3-4 pair, with the same connectors as LT3-4, forms large arrays that can span almost 1 μm and can involve more than 500 tectosquares. The plain central cavity of ST3 and ST4, too small to be well resolved by AFM, gives a striped velvet texture to these assemblies (Fig. 3; fig. S3). STs and LTs can also be combined when in the trans configuration. For example, the mix of ST3 and LT4 forms basket weave patterns characteristic of the LT and ST alternating arrangement (Fig. 3).

More complex patterns can be obtained from a greater number of tectosquares by using additional tail connectors or by preventing association at specific positions within the 2D lattice. Both LT7-8-9 and LT10-11-12-13-14 sets take advantage of six connectors to form the lace and polka dot patterns, respectively (Fig. 3 and fig. S3). For LT10-11-12-13-14, the regularly spaced dotted motif is programmed with five LTs assembling symmetrically in an all trans configuration network, two of them lacking one of their tails.

Tectosquare assembly is highly dependent on the directionality of the 3′ tail (Fig. 1F). We observed significantly larger architectures with type I LT networks than with type II (fig. S4, A and B) (*28*). Nevertheless, patterns taking advantage of eight connectors with various 3′ tail orientations can be obtained. The tartan pattern LT7-8-15-16 is derived from the lace pattern LT7-8-9 (Fig. 3A), by replacement of LT9 with LT15-16, a tectosquare dimer formed by association of type III and type IV LTs (Fig. 1F). The different directionality of LT15 and LT16 tails leads to the formation of a lattice with meshes of 28 ± 3 nm, twice as big as those obtained with LT5-6 and LT7-8-9 (Fig. 3B). LT17-18-19-20, a set of four type V tectosquares with 3′ tails pointing in the four cardinal directions, assembles into the cross pattern corresponding to a C_4 pseudosymmetrical arrangement of LTs (Fig. 3A). This regular lattice has two distinct square-shaped mesh sizes of 12 ± 2 nm and 17 ± 2 nm that correspond to the LT structure and the central hole formed by association of four LTs, respectively (Fig. 3B).

As an initial step toward fully addressable self-assembling materials, we designed three sets of tectosquares to assemble specifically into finite aperiodic nanogrids (Fig. 4; fig. S4). To control the size and shape of the RNA assembly, some LTs lacking a 3′ tail at specific corners are programmed to act as edges. The 2 by 2 grid is a cross of 45 nm formed of four type V LTs linked by four different connectors. The 3 by 3 and 4 by 4 grids are symmetrical, modular arrangements of five and eight different type I LTs, respectively. In the 3 by 3 grid, four edge LTs are linked to a central LT by six different connectors. In the 4 by 4 grid, six edge LTs assemble around a central 2 by 2 cross of two LTs through 12 different connectors. According to our structural models, the 2 by 2 grid is not perfectly flat. This partially explains its infrequent observation on the mica surface and the rhombus, rather than square, shape adopted by the 2 by 2 LT during the AFM imaging process (Fig. 4). By contrast, more than a hundred diamond-shaped 3 by 3 grids are identified on a 16-μm^2 surface, in perfect agreement with the flat planar arrangement expected for type I LTs (Fig. 4; fig. S4, C and D). The 4 by 4 grid demonstrates that the assembly of up to 27 different tectoRNAs can be hierarchically and reproducibly controlled to form RNA nanoscale jigsaw puzzles, which suggests that aperiodic assemblies of even greater molecular diversity can be obtained with additional connectors (*29*).

We have demonstrated that two rRNA structural motifs participate in a predictable manner to stabilize, position, and pack RNA helices without the need of proteins. The length and geometry, rather than the sequence of loops, predispose the formation of linear coaxial stacks of helices in KL complexes. Similarly, it is the bent geometry of the RA motif that favors stacking of the 3′ end tail connector in continuity with its 3′ stem. The importance of base stacking is emphasized by the inability of unstacked dangling 5′ tail connectors to form any organized networks. Thus, in both the ribosome and tectosquares, RA and KL motifs are likely to assist the assembly process not only by local contributions to a specific RNA fold but also by reducing the entropy cost.

The subtle interplay of enthalpy and entropy that successfully promotes the formation of tectosquare assemblies is highly dependent on the strength, length, and orientation of the tail connectors and the environmental cues (RNA and divalent ion concentrations, temperature, and assembly protocols). For instance, thermodynamically stronger tail connectors of the same order of magnitude as KL complexes, as well as a reduced initial temperature of assembly, can negatively contribute to ordered assembly by kinetically trapping tectosquares in wrong configurations. Moreover, variation of the magnesium concentration can be used to switch on and off tectosquare assembly. Understanding phase diagrams for assembly is thus important for finding annealing conditions to self-heal irregular lattice points. Tectosquares assemble into their respective lattices at nanomolar concentrations, where-

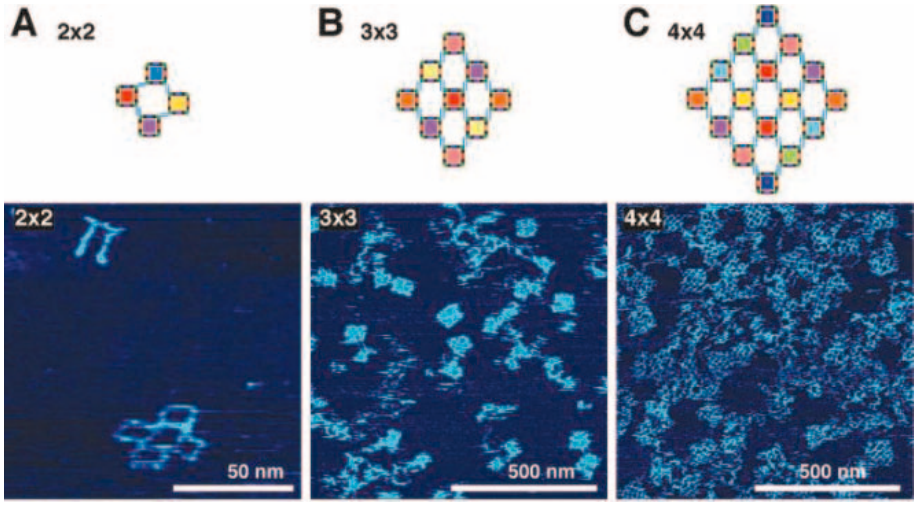

Fig. 4. Tectosquare nanogrids of predefined size, shape, and molecular composition. Schematic and AFM images under solution for (**A**) 2 by 2 (LT21-24), (**B**) 3 by 3 (LT10-12, 25-26), and (**C**) 4 by 4 (LT27-34) RNA nanogrids.

as K_d values measured for single 3′ tail connectors are in the micromolar range. This fact and the small number of overlapping RNA lattices observed by AFM suggest that epitaxial phenomena occurring between RNAs and magnesium ions adsorbed on the negative mica surface might promote assembly. The observed RNA networks grow in a radial fashion. All LT tectosquare arrays involve a similar number of molecules, indicating that LT assembly is independent of the nature of the pattern formed (Fig. 3). However, STs generate significantly larger lattice networks. Despite their apparent robustness and stiffness, multiple AFM scans can disrupt the edges or deplete one or two tectosquares within the lattice. It is clear that, as a soft matter medium, the stability and size of RNA networks can still be improved and their visualization by AFM still remains challenging.

This work offers an attractive alternative to DNA, protein, and synthetic molecules for directed arrangement of matter at a molecular level (*1*, *30*–*33*) and lays the foundation for generalization to periodic 3D nanomaterials of RNA. As fully addressable, programmable assemblies, tectosquare jigsaw puzzles can serve as hosts to organize at relative defined positions various molecular components with high precision and to generate nanochips, nanocircuits, and nanocrystals with potential applications in nanotechnology and material sciences (*1*, *32*). With its underlying modular and hierarchical construction displaying a minimal set of primitive operations, the tectosquare system could possibly be a Turing-universal computing molecular system (*10*, *34*, *35*). It can also be a valuable tool for studying self-organization and emergence of complexity out of randomness (*35*). For instance, an unanswered question is whether combinatorial population of tectosquares could still assemble accurately into organized architectures.

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23. Considering that each of the four tectosquare units can either have no tail, or a 3′ tail of *n* different sequences with different size (*s*) and orientation (*o*), a total of $4(son + 1)$ different a, b, c, and d tectoRNAs can be combined to construct $(son + 1)^4$ tectosquares.
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28. LTs associate better when tails are oriented parallel to their a-d and b-c sides. This fact supports our twofold symmetrical models (Fig. 1C; fig. S1), with a-d and b-c being parallel to each other and a-b and c-d being tilted. Assembly through tails oriented parallel to a-b and c-d is nevertheless possible.
29. With 12 different tail-tail connectors, we are presently able to generate a 3 by 3 grid made of nine different tectosquares with the position of each of the 36 constitutive tectoRNAs being fully addressable within the RNA lattice.
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36. This paper is dedicated to St. Joseph, patron saint of carpenters, and to F. Michel and E. Westhof, L.J.'s mentors. Thanks to S. and L. Baudrey for technical assistance and to C. Geary, H. Waite, and S. Parsons for critical reading of the manuscript. A.C. thanks the Department of Bioorganic Chemistry, Polish Academy of Sciences, CM&MS, Lodz, 90363, Poland. Funding for this work was provided by faculty start-up funds from UCSB to L.J. and by grants from NSF to L.J. (CHE-0317154 and MRSEC DMR00-80034) and H.H. (MCB0236093).

Supporting Online Material

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Translation of DNA Signals into Polymer Assembly Instructions

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We developed a DNA nanomechanical device that enables the positional synthesis of products whose sequences are determined by the state of the device. This machine emulates the translational capabilities of the ribosome. The device has been prototyped to make specific DNA sequences. The state of the device is established by the addition of DNA set strands. There is no transcriptional relationship between the set strands and the product strands. The device has potential applications that include designer polymer synthesis, encryption of information, and use as a variable-input device for DNA-based computation.

We built a DNA nanomechanical device that mimics the translational capabilities of the ribosome. In response to a DNA signal, it aligns a series of molecules in specific positions; these molecules are then fused together in a specific order. For convenience, we have prototyped this system with DNA, so the products are DNA oligonucleotides of a defined sequence. Thus, in this case, the chemistry of the product is similar to that of the signal molecules, but there is no complementary relationship to the signal sequences. By using DNA molecules to set the states of two DNA PX-JX_2 devices (*1*) independently, we programmed the synthesis of four different product molecules.

The PX-JX_2 device is a sequence-dependent DNA machine, the state of which is controlled by hybridization topology (*1*). It can assume two structural states (termed PX and JX_2), which differ from each other by a half-turn rotation of one end of the molecule relative to the other end (Fig. 1A). Two different pairs of set strands can bind to the framework of the device, thereby establishing which structural state it adopts. The set strands contain short unpaired segments ("toeholds") at one end to facilitate their removal by unset strands that bind to the toeholds and then remove the set strands by branch migration (*2*). In addition to the PX-JX_2 device, numerous variants of sequence-dependent control, pioneered in DNA tweezers by Yurke *et al.* (*2*), have been reported; these include a DNA

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actuator (*3*), a three-state device (*4*), and a DNA bipedal walking machine (*5*). Neither these nor other shape-shifting DNA devices (*6*) have been incorporated into a larger context that performs a useful task. By contrast, we used the structural state of the device reported here for positional control of the products of polymer concatenation.

We incorporated two PX-JX_2 devices in succession, thereby controlling the relative orientations of a diamond-shaped motif (*7*) and a pair of double-diamond-shaped wings (Fig. 1B). The Arabic numerals in Fig. 1B label individual sticky ends; these sticky ends are available to bind DNA double-crossover (DX) molecules (*8*) that contain a continuous DNA strand extending from one end to the other. This continuous strand ultimately will be a component of the product. The rest of the DX molecule plays a role analogous to that of tRNA in translation: It serves as an adaptor between the strand that it carries and the device. The set strands of the device contain the signal, or message, that configures the sticky ends to bind one of a pair of DX molecules in each of the two gaps.

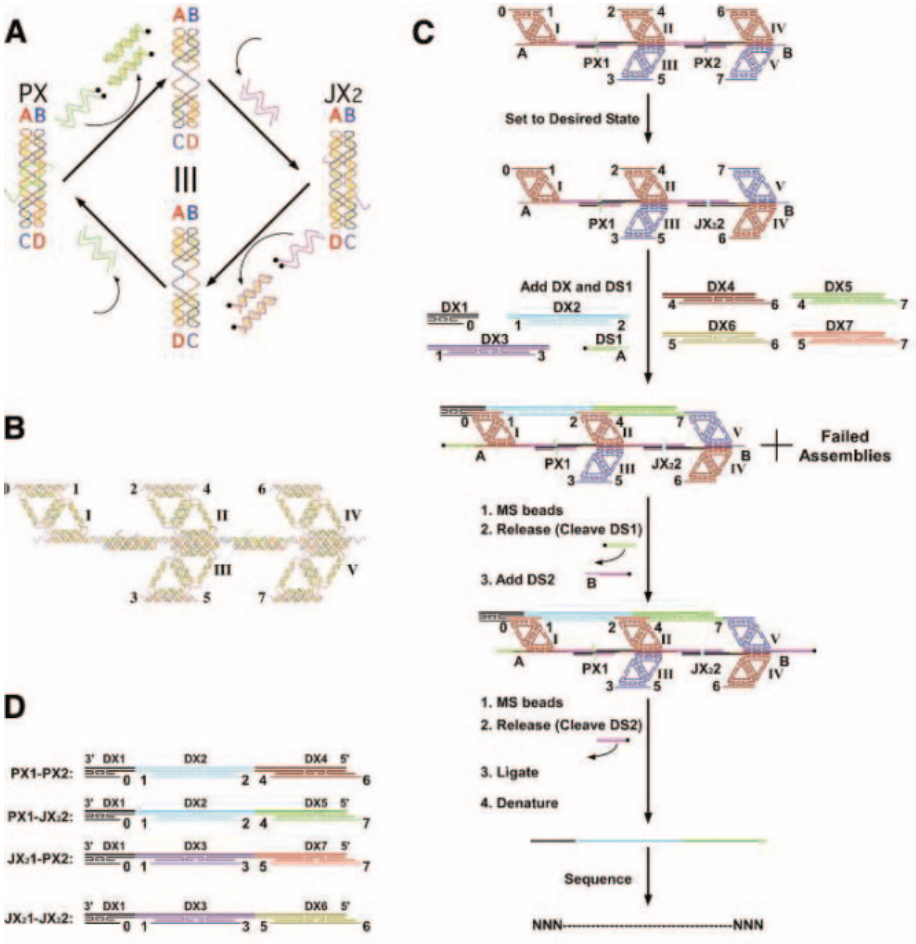

Fig. 1. Schematic drawings of the system and its components. (**A**) The PX state of the device is shown at the left, with green set strands. These are removed by biotinylated (black dots) unset strands to leave a naked frame (top). Adding the purple set strands puts the device in the JX_2 state. The bottom of the cycle shows restoration of the PX state. The two states differ by a half-turn rotation, as highlighted by the letters A, B, C, and D flanking the helices. (**B**) Five diamond motifs (*7*) are labeled by Roman numerals, and the sticky ends are labeled by Arabic numerals. The diamonds are connected to form double-diamond wings by means of a PX linkage, to give the wings dyad symmetry (*14*). There is an initiator-diamond motif at the left (I), and two double-diamond motifs are at the center and right. The initiator diamond and the double diamonds are connected by PX-JX_2 devices, so the relative orientations of the sticky ends can be varied. (**C**) The initial assembly of the device is shown at the top, similar to the structure in (B). Diamonds III and IV have been drawn with different colors for clarity. A and B indicate sticky ends used in purification. The next step entails setting the state of the device. The DX molecules are all added to the solution, along with an initiator DX1 (black) and a biotinylated double strand, DS1, complementary to A. After left-side purification by magnetic streptavidin (MS) beads, and release by cleaving DS1, right-side purification is achieved similarly with DS2 and the addition of magnetic beads again. The purified complex is then ligated, and the continuous strand is denatured, purified, and sequenced. (**D**) The four ligated DX molecules are shown with the same color coding as in (C). The continuous strands across the top are the final products that are sequenced.

Figure 1C illustrates the flow chart for the experiments we performed. First, the device is constructed with both PX-JX_2 machines in the PX state. Unset strands,

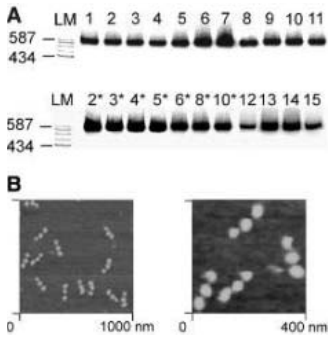

Fig. 2. (**A**) This is a 3.5% nondenaturing gel of the complex, composed of all 22 strands. LM indicates linear markers. Each of the strands [numbering in (9) and figs. S1 to S3] is labeled separately with radioactive phosphate. The number above each lane shows the labeled strand. In each case, the strand is incorporated cleanly into the complex, with no doubling and no breakdown products evident. (**B**) The AFM images were prepared as described previously (*1*). The DX molecules were omitted for clarity in the image. The left image is a large field, and the right image is a zoom. The single- and double-diamond nature of the complex is evident from the small-large-large nature of the sample molecules. It is clear that the purification protocol used is quite successful at eliminating failure products from the sample.

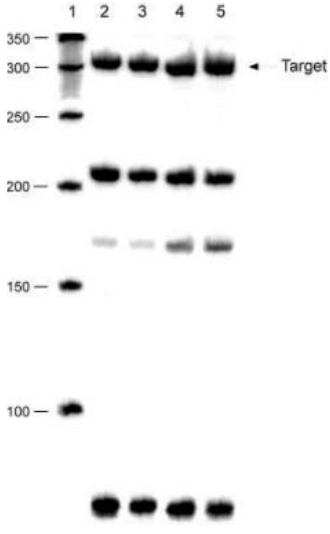

Fig. 3. This is a 6% denaturing gel autoradiogram showing the products of the ligation. The first lane contains a 50-nucleotide ladder. Lanes 2 to 5 contain, respectively, the products of setting the device states to PX-PX, PX-JX_2, JX_2-PX, and JX_2-JX_2. The black strand (Fig. 1D) is labeled in each case. The target band is prominent in all lanes, although failure products are evident. The band between 150 and 200 may be an erroneous product; it represents 0.3%, 0.2%, 1.0%, and 1.2%, of the material in lanes 2 to 5, respectively.

followed by specific set strands, are then added to the device, setting its state. The complete set of DX molecules is added to the solution, and the correct ones (cyan and green in Fig. 1C) bind in the correct sites between the diamond structures, as dictated by the sticky ends. In addition, an "initiator" DX (black in Fig. 1C) is bound on the top of the leftmost double diamond. After ligation, the DX molecules are dissociated, the target strand is isolated, and its sequence is determined. Its target length is longer than any other strand in the system, so it is easy to isolate from failure products and fortuitous molecules that bind to the opposite (bottom) side of the device. Figure 1D shows the way that the selection of set strands directs the synthesis of different products, in the same way that different mRNA molecules direct the synthesis of different polypeptide chains. Experimental methods are described in (*9*).

Figure 2A shows a nondenaturing gel in which each of the 22 strands of a wing component is labeled individually. The uniform nature of the gel demonstrates that the complex contains all of the strands and that no strand partially denatures from it. Atomic force microscopy (AFM) images (Fig. 2B) show purification of the device in the absence of DX molecules. Each molecule contains two large components, corresponding to the double-diamond wings, and a smaller one on the end, corresponding to the single diamond that supports the initiator DX. The product strands from the four different combinations are shown in a denaturing gel in Fig. 3. Numerous ligation failures are evident, but the target molecules containing 307 nucleotides are well represented on the gels. In each case, the target molecule contains the expected sequence. The designed sequences of the device components and the set strands, the experimental sequences of the products, and their sequencing traces are shown in figs. S4 to S7.

We produced a device that translates a DNA signal into an unrelated sequence. The connection between the signals and the products (the "genetic code" for this system) has been established arbitrarily, so that there is no transcriptional relationship between them. It is evident that this simple device prototypes an arbitrary, but general, encryption method (*10*). In addition, this type of device has been suggested as the basis for a finite-state machine with variable input, whose output can be used for universal computation (*11*). From a chemical standpoint, we expect to be able to couple this system with a recent method that adds reactive groups to the backbone residues of nucleotides; that method enables the attachment of arbitrary polymers to the DNA (*12*). Adding a reactive group to the continuous chain at a few accessible sites (e.g., once per helical turn) would be independent of steric effects. Such groups could be used in this system to scaffold the construction of diverse and unprecedented polymers of well-defined size and composition. This device lacks the translocational capability of the ribosome, so that product length is similar to device size; nevertheless, translocation is well within the scope of DNA nanotechnology (*13*), suggesting that it can be incorporated in future versions of the device.

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A Late-Transition Metal Oxo Complex: $K_7Na_9[O{=}Pt^{IV}(H_2O)L_2]$, $L = [PW_9O_{34}]^{9-}$

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Terminal mono-oxo complexes of the late transition metal elements have long been considered too unstable to synthesize because of repulsion between the oxygen electrons and the mostly filled metal d orbitals. A platinum(IV)-oxo compound flanked by two polytungstate ligands, $K_7Na_9[O{=}Pt(H_2O)L_2]$, $L = [PW_9O_{34}]^{9-}$, has now been prepared and isolated at room temperature as air-stable brown crystals. X-ray and neutron diffraction at 30 kelvin revealed a very short [1.720(18) angstrom] Pt–O bond and no evidence of a hydrogen atom at the terminal oxygen, ruling out a better precedented Pt–OH complex. Density functional theory and spectroscopic data account for the stability of the Pt(IV)-oxo unit by electron withdrawal into delocalized orbitals of the polytungstates.

Despite the high electronegativity of oxygen, the terminal oxo ligand is a strong π electron donor. Lone oxygen atoms therefore bind most stably to high valent early transition metals, such as Ti(IV), V(V), and their heavier congeners. In these complexes, electrons can delocalize from oxygen into the vacant d orbitals on the metal. Thus, d^0 to d^2 oxo compounds are ubiquitous, and the oxo ligand can be ancillary to reactions in the coordination sphere of the metal. Moving from left to right across the periodic table, the d orbitals fill with valence electrons, and oxo ligands are destabilized by repulsion (*1*–*5*). There are few stable d^4 metal oxo complexes, a hydrogen-bonded d^5 complex (*6*), and only a single reported d^6 oxo, the $NaRe(O)(PhCCPh)_2$ complex isolated by Mayer and co-workers (*7*). For iron and the later transition metals, even monomeric hydroxo compounds are rare.

At the same time, the instability of the late metal oxo linkage proves useful in

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catalysis. Iron-oxo intermediates are invoked in numerous enzymatic oxidations, both for heme-based oxidases (*8–10*) and non-heme oxygenases (*11–17*). Similarly, transient oxo intermediates are thought to play a major role in O_2 activation at platinum surfaces, whether in automobile catalytic converters (*18*), fuel cells (*19*), or industrial catalysis (*20*). Efforts to model such intermediates have been hampered because, in the absence of an oxygen acceptor, O-coordination to these electron-rich metals leads to disproportionation or to cluster formation in order to diminish the electron repulsion. We reasoned that a stable platinum oxo complex should therefore require an electron-accepting ligand framework.

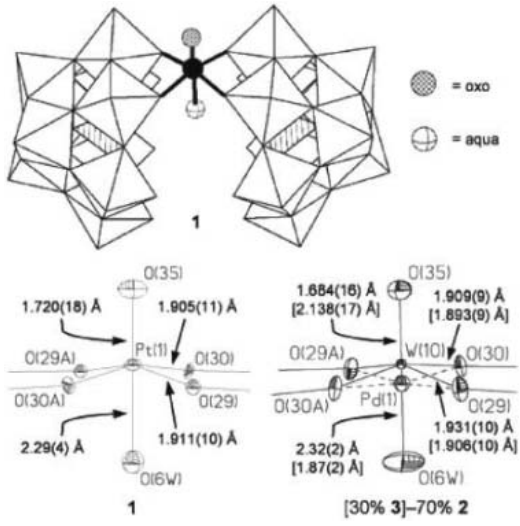
Polyoxometalates (POMs) are metal-oxygen anionic clusters that can function as multidentate, totally inorganic, and oxidatively resistant ligands for redox-active ions (*21*, *22*). POMs have acidities, redox potentials, polarities, solubilities, and other molecular properties that can be systematically altered. In addition, the delocalized nature of the molecular orbitals in POMs renders them highly effective π-acceptor ligands. Although a few platinum-containing POMs are known, none of them have been structurally characterized to date. The Pt(II) salt K_2PtCl_4 reacts with the polytungstate cluster A-α-$Na_9PW_9O_{34}$ (*23*) in H_2O. Rapid KCl addition leads to the kinetic precipitation of a white solid, which the infrared and ^{31}P nuclear magnetic resonance (NMR) data suggest is $[Pt^{II}(PW_9O_{34})_2]^{16-}$. This was redissolved in fresh H_2O at 55°C. During a 3-day exposure to air, the solution turned yellow, and brown crystals of $K_7Na_9[Pt(O)(H_2O)(PW_9O_{34})_2]\cdot 21.5H_2O$ (**1**) emerged in 25% yield (*24*). This formulation was rigorously confirmed by elemental analysis (*25*). The identical protocol under argon, rather than air, yielded no product. Compound **1** was thermally stable to ~425°C, at which point thermogravimetric analysis and differential scanning calorimetry showed decomposition.

An x-ray diffraction study of crystalline **1** at 193 K confirmed a platinum-oxo linkage (Scheme 1). The very short [1.720(18) Å] Pt=O bond is trans to a longer [2.29(4) Å] Pt–OH_2 bond. Known Pt(IV)–OH bond lengths from 50 structures in the Cambridge Structural Database range from 1.943 Å to 2.079 Å and average 1.998 Å. Two symmetrically equivalent polytungstate clusters each bind to the central platinum through two oxygens, defining a square equatorial plane in an approximate C_{2v} molecular geometry. All Na^+ and K^+ counterions were located in the analysis, further confirming our assigned formulation.

Given the lack of precedent for late-transition metal oxo compounds, we acquired two additional x-ray diffraction data sets at lower temperatures (100 K and 30 K) to improve the precision in the atomic positions. After growing sufficiently large crystals (1 by 2 by 3 mm^3), we also studied the structure by neutron diffraction (Fig. 1), which is particularly sensitive to the presence of hydrogen atoms. The structures derived from all of these data sets were consistent with a platinum-oxo; there is no evidence of hydrogen bound to the terminal oxygen to give a better precedented hydroxo complex.

A colorless d^0 analog to compound **1** in which tungsten replaces platinum is known: $[(O)W(H_2O)(PW_9O_{34})_2]^{14-}$ (**2**) (*26*). The W≡O bond (1.67 Å) is slightly shorter than the corresponding Pt=O bond in **1**. For a d^2 configuration, the average bond lengths for W(IV)=O, Re(V)=O, and Os(VI)=O are 1.72(6) Å, 1.67(2) Å, and 1.70(3) Å, respectively. For a d^3 configuration, the average bond lengths for W(III)=O and Re(IV)=O are 1.72(3) Å and 1.74(2) Å, respectively. No data for d^3 Os(V)=O were found. The d^6 Re(I) complex reported by Mayer has a Re(I)=O bond distance of 1.756 Å. Given the fact that Pt(IV) and Re(I) are isoelectronic, one would expect the Pt(IV) to have a smaller radius, because it has a higher nuclear proton count and higher effective nuclear charge than Re(I). This would lead to a shorter bond length for Pt(IV)=O than for Re(I)=O.

In both **1** and **2**, the central metal atom is displaced out of the O_4 equatorial plane toward the terminal oxo (by 0.31 Å for platinum and 0.45 Å for tungsten). This dome-like geometry is a nearly universal structural feature in metal-oxo moieties (*22*, *27–29*). If the pH is lowered from 7 to 2, the reaction conditions used to prepare **1** can lead to formation of the tungsten oxo complex through decomposition of the polytungstate ligands. The x-ray data and elemental analysis showed no evidence for contamination of isolated **1** with this side product. Attempts



Scheme 1.

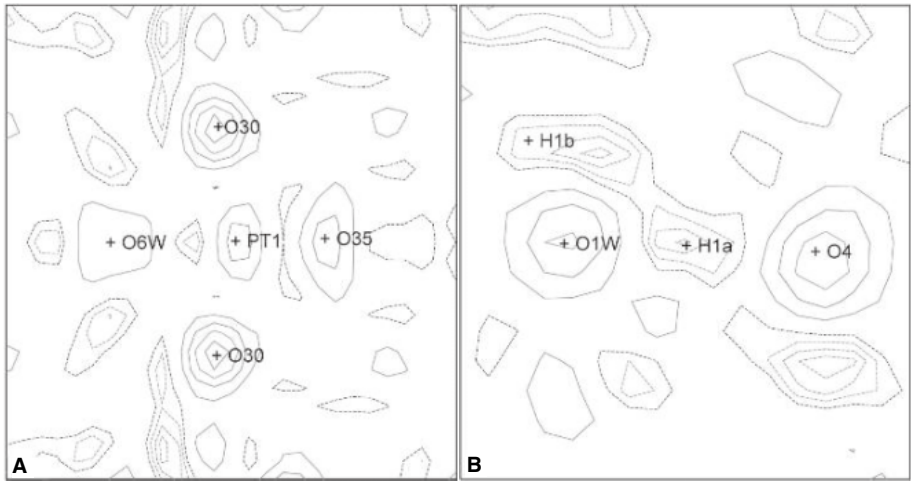


Fig. 1. F_{obs} Fourier maps derived from single crystal neutron time-of-flight Laue diffraction data taken at 30 K. Data were collected on the single crystal diffractometer at the Intense Pulsed Neutron Source, Argonne National Laboratory. Atom positions in the model are marked with a + and are labeled with their names. In this sample, all non-hydrogen atoms have positive scattering lengths (solid contour lines) and only hydrogen atoms have negative scattering lengths (dashed lines). (**A**) Fourier map of the Pt-O35-O6W-O30-O30* region of **1** (see Scheme 1 for the structural orientation). Low-level negative contours located near O35 were not able to be modeled in a chemically reasonable fashion. This map size is 8 Å on an edge. (**B**) Fourier map showing the free lattice water O1W-H1a-H1b plane and a nearby O4 (POM) atom. This map is typical of those seen for the water molecules that could be well modeled. H1a is clearly bound to O1W and appears to be hydrogen-bonded to O4 at a distance of 1.52 Å. An additional unassigned negative scattering region is seen close to O4 and may denote a hydrogen atom associated with neighboring water oxygen atoms. No such feature is seen close to O35 (Pt-oxo) in (A). This map size is 5 Å on an edge.

to prepare a Pd(II) analog of **1** in water were unsuccessful, because a cocrystalline mixture of **2** and $[Pd^{II}(H_2O)_2(PW_9O_{34})_2]^{16-}$ (**3**) (Scheme 1, lower right) was obtained (*30*). A Raman spectrum of **1** displays the identical W–O and P–O peaks of **2**, but no peak for the Pt=O stretch is observable (*24*).

With the composition and physical structure of **1** confirmed, we turned to the electronic structure of this compound. Charge balance considerations supported a +4 oxidation state at platinum, corresponding to a d^6 valence configuration. Room temperature susceptibility studies showed a diamagnetic ground state, consistent with d^6. The diamagnetism is further confirmed by the ^{31}P NMR spectrum (single peak at –8.8 ppm, peak width at half height $\Delta\nu_{1/2}$ = 30 Hz) (*31*).

The d orbitals of a local-C_{4v}-symmetry $O{=}PtO_4(H_2O)$ unit, in order of increasing energy, are d_z^2 (bonding), $d_{xz,yz}$ (bonding), d_{xy}, $d_{xz,yz}$ (antibonding), $d_{x^2-y^2}$, and d_z^2 (antibonding) (Fig. 2) (*2*–*5*). Typically, the strong ligand field perturbation of the terminal oxo leads to a large destabilization of the $d_{xz,yz}$ orbital pair, because of π-type antibonding interactions, and a severe destabilization of the metal d_z^2 orbital from σ-type antibonding interactions.

The electronic spectrum of **1** has three bands, at 574 nm (band 1), 502 nm (band 2), and 428 nm (band 3), with extinction coefficients of 43, 73, and 210 M^{-1} cm^{-1}, respectively, as well as the intense oxygen-to-tungsten charge transfer bands (absorbance λ < 400 nm) characteristic of all polytungstates (*22*). Analysis of the electronic absorption spectra of **1** is consistent with tentative assignments for the Pt(IV) ligand field bands as $e \rightarrow b_1$ (bands 1 and 2) and $b_2 \rightarrow b_1$ (band 3) (table S1). Bands 1 and 2 derive from low-symmetry and spin-orbit splitting of the E excited state ($A_1 \rightarrow E$ transition). Band 3 ($A_1 \rightarrow A_2$ transition) is a symmetry forbidden transition in the local C_{4v} symmetry and is likely allowed by a combination of the C_{2v} distortion and mixing of high intensity charge transfer states.

Importantly, this assignment results in a d_{xy}—$d_{xz,yz}$ orbital splitting of only ~5000 cm^{-1} (*32*), which is highly reduced from the 12,000 to 16,000 cm^{-1} splitting expected for $d^{1,2}$ metal-oxo systems (*3*, *4*). The reduced splitting can be explained by Pt $d_{xz,yz} \rightarrow$ W $d_{xz,yz}$ back-bonding interactions mediated by the bridging oxo groups (*33*). Here, the empty W(VI) orbitals of e symmetry play a π-acceptor role, lowering the energy of the Pt $d_{xz,yz}$ orbital set and reducing their net antibonding character. This effect has been previously observed in high-valent iron-oxo systems where the equatorial porphyrin ligand plays a π-acceptor role with respect to the Fe $d_{xz,yz}$ orbitals (*2*). As in this previous case, the effect of the back-bonding interaction is to strengthen the Pt–O bond by partially delocalizing the Pt(IV) $d_{xz,yz}$ electron density onto the polytungstate portion of the compound.

Because the $d_{xz,yz}$ (π*) orbitals are completely filled, the terminal platinum-oxo bond possesses no conventional π-bonding character and is formally a single bond. However, this strong Pt $d_{xz,yz} \rightarrow$ W $d_{xz,yz}$ back donation and concomitant stabilization of the Pt $d_{xz,yz}$ orbitals results in the following properties of the platinum-oxo bond: a net increase in π-bonding character, a bond order effectively higher than 1, a shorter Pt–O distance (1.72 Å) than typical Pt–O single bonds (*34*), and a non-basic terminal oxo oxygen. Many terminal oxo species with a formal single bond to oxygen and high electron density on the terminal O, such as *N*-oxides and metavanadate, are not protonated at neutral pH. Computational studies of model $O{=}Pt(H_2O)(POM)_2$ systems of varying complexity {from $[PtO]^{2+}$ to $[H_4PtO]^{2-}$ and $[(POM)_2PtO]^{n-}$} support these conclusions and indicate that the electronic structure of **1** is strongly affected by the electron accepting capacity of the polytungstates. The application of the various density functional theory and multideterminant-based CASSCF and CASPT2 approaches shows that inclusion of a ligand field (upon going from $[PtO]^{2+}$ to $[H_4PtO]^{2-}$) shifts the $d_{x^2-y^2}$ orbital to higher energy than the two antibonding d_{xz}(PtO) and d_{yz}(PtO) orbitals. As a result, the diamagnetic state with the $(d_{xy})^2(d_{xz})^2(d_{yz})^2(d_{x^2-y^2})^0$ orbital configuration becomes the lowest in energy. Inclusion of the POM ligands reduces the antibonding nature of the d_{xz}(PtO) and d_{yz}(PtO) orbitals and also slightly stabilizes them.

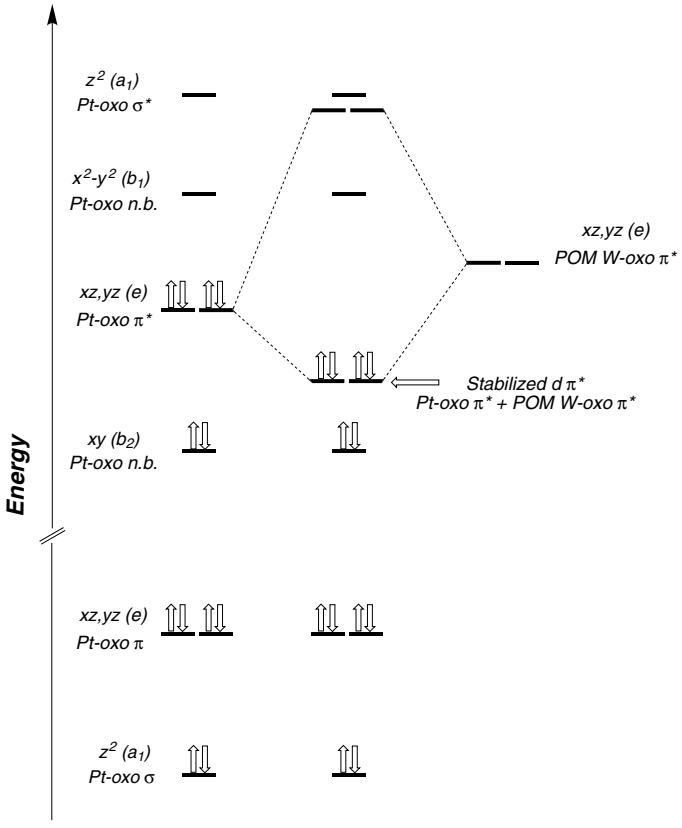


Fig. 2. Simple molecular orbital diagram for the Pt(IV)-oxo portion of **1**, illustrating the stabilization resulting from Pt $d_{xz,yz}$—W $d_{xz,yz}$ interaction and effective donation of electron density from the formal Pt-oxo π* orbitals to delocalized orbitals on the polytungstate ligands. The d orbitals of the metal-oxo unit are shown with their corresponding C_{4v} symmetry labels. Energy ordering for the unperturbed Pt-oxo orbitals (left) are those appropriate for typical metal-oxo species and indicate the large destabilization of the metal $d_{xz,yz}$ (π*) and d_z^2 (σ*) orbitals due to antibonding interactions with the terminal oxo. For low d-electron counts (d^{0-2}) the metal-oxo bond order is three, because the d_{xy} orbital is nonbonding with respect to terminal metal-oxo bonding interactions. However, for d^{3-6} electron counts, each additional electron reduces the bond order by 0.5, weakening the metal-oxo bond and potentially activating the oxo ligand.

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25. The elemental compositions of the bulk sample and the single crystals analyzed by x-ray and neutron diffraction are effectively identical. Calculated elemental analysis for bulk sample of $K_7Na_3Pt^{IV}O(H_2O)(PW_9O_{34})_2{\cdot}21.5H_2O$: K, 4.92; Na, 3.72; P, 1.11; Pt, 3.51; W, 59.47. Found: K, 4.87; Na, 3.66; P, 1.12; Pt, 3.71; W, 60.71; Molecular weight, 5564 g/mol.
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30. Although **2** and **3** cocrystallize, a good x-ray structure (R = 5.2%) (*24*) of a crystal 70% **2** and 30% **3**. It clearly distinguishes the positions of the central W(VI) and Pd(II) centers and the short [1.684(16) Å] W^{VI}-oxo bond from the long [2.138(17) Å] Pd-OH_2 bond (Scheme 1, lower right).
31. Line width and chemical shift data from ^{31}P NMR have been shown to be highly sensitive to the presence of paramagnetic metal centers within the polytungstate framework (*36*).
32. The large electron-electron repulsion in the A_1 ground state also contributes to the apparent d_{xy}—$d_{xz,yz}$ orbital splitting. A larger d_{xy}—$d_{xz,yz}$ splitting may result in low-energy one-electron promotions to d_{z^2}.
33. It was previously proposed in $[Ru^{II/III}PW_{11}O_{39}]^{n-}$ based on ^{183}W NMR and the presence of metal-to-ligand charge transfer bands that the polyoxometalate ligand in this complex displays π acceptor properties (*37*).
34. The average Pt(IV)-O single bond distance is 1.992 Å (based on an average of 10 different structures). This bond is defined as being Pt(IV)-O-X, where X is not H.
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38. We thank J. Reibenspies at Texas A & M University for collecting the low temperature x-ray data set and I. A. Weinstock for useful discussions. Supported by the U.S. Department of Energy (DOE) (C.H., J.M., and K.M.), the NSF (grant no. CHE-0236686 to C.H.), and the NIH (grant no. GM-057378 to M.L.K.). The work at Argonne National Laboratory was supported by the DOE Office of Basic Energy Sciences (contract no. W-31-109-ENG-38). Additional details on the crystal structure investigations may be obtained from the Fachinformationszentrum Karlsruhe, 76344 Eggenstein-Leopoldshafen, Germany, on quoting registry nos. CSD-414500 and CSD-414522 (neutron) for **1** and CSD-414501 for **2** and **3**.

Supporting Online Material

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Materials and Methods
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Clues from Fe Isotope Variations on the Origin of Early Archean BIFs from Greenland

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Archean rocks may provide a record of early Earth environments. However, such rocks have often been metamorphosed by high pressure and temperature, which can overprint the signatures of their original formation. Here, we show that the early Archean banded rocks from Isua, Akilia, and Innersuartuut, Greenland, are enriched in heavy iron isotopes by 0.1 to 0.5 per mil per atomic mass unit relative to igneous rocks worldwide. The observed enrichments are compatible with the transport, oxidation, and subsequent precipitation of ferrous iron emanating from hydrothermal vents and thus suggest that the original rocks were banded iron formations (BIFs). These variations therefore support a sedimentary origin for the Akilia banded rocks, which represent one of the oldest known occurrences of water-laid deposits on Earth.

In studies of early life and ancient environments on Earth, geological context is of key importance but can be difficult to ascertain (*1*). Ambiguities in interpretations of the early Earth record are mainly caused by the high grade of metamorphism that all remnants of early Archean supracrustal rocks have experienced during burial, which overprints the primary characteristics of the rocks. One example of this ambiguity concerns the origin of a banded quartz-pyroxene rock formation on Akilia Island, where trace elements and field relationships have been used to argue either for (*2–6*) or against (*7–9*) a sedimentary origin. Because recrystallization and metasomatism have partially influenced the trace element distribution in these rocks, it is important to identify additional markers, such as S (*10*) or Fe (*11*) isotopes, that may have retained an original premetamorphic signature.

The Fe isotopic composition, F_{Fe}, which is expressed as per mil per atomic mass unit (‰/amu) deviation relative to the composition of a reference material (*12*, *13*), is affected by biological processes and can potentially be used to trace life in present and past environments (*11*, *14–17*). However, various abiotic processes that operate in aqueous fluids at low temperature are known to fractionate Fe isotopes, compromising to some extent the use of F_{Fe} as a biosignature (*11*, *14*, *15*, *18*, *19*). A notable feature of Fe isotope systematics is the near-constant F_{Fe} of igneous rocks formed at different times, locations, and tectonic settings (Fig. 1A and Table 1) (*12*, *15*, *20–23*). In contrast, chemical sediments and, more specifically, BIFs are known to have variable F_{Fe} values (Fig. 1A) (*24*), which raises the possibility that Fe isotopes can be used to infer the nature of the protolith of heavily metamorphosed rocks (*11*). To address what might be the oldest sedimentary sequence on Earth, we have analyzed the F_{Fe} of igneous and banded rocks from Isua, Akilia, and Innersuartuut, Greenland (Table 1).

Powdered rock samples were digested in acid (HF-HNO_3-$HClO_4$). Iron was then purified on an ion exchange resin, and its isotopic composition was analyzed with a multicollector inductively coupled plasma mass spectrometer (Table 1) (*12*). The ultramafic and gabbroic samples SM/GR/97/3, SM/GR/97/4, and SM/GR97/6 were found directly in contact with the banded quartz-pyroxene rocks in question (*2–9*) in the Akilia association on the southwestern end of Akilia Island. Samples SM/GR/97/2 and SM/GR/97/7 are part of the Amîtsoq gneisses that directly surround the Akilia association. The igneous rocks from Akilia (F_{Fe} = 0.022 ±

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0.050 ‰/amu; $n = 5$ measurements) (Table 1 and Fig. 1A) have the same F_{Fe} values within uncertainties as worldwide igneous rocks ($F_{Fe} = 0.040 \pm 0.009$ ‰/amu; $n = 76$) (Table 1) (*12*, *15*, *20–23*). This result demonstrates that the F_{Fe} of the silicate portion of Earth available for sampling did not change drastically between the early Archean era and the present day. It also indicates that if Fe was mobilized during metamorphism, its isotopic composition was not modified at the bulk sample scale.

BIFs have variable F_{Fe} values. This is best demonstrated by the BIFs from the Kaapvaal Craton (2.5×10^9 years old) (*24*). As illustrated in Fig. 1, the F_{Fe} of this formation spans values from –1.20 to +0.57 ‰/amu with a strong mineralogical control (from isotopically light pyrite and Fe-carbonate to isotopically heavy hematite and magnetite). We have analyzed the F_{Fe} of mineral separates and bulk rock samples of a BIF from Isua (IF-G, iron formation geostandard), quartz-pyroxene rocks from Akilia [G91-26, also referred to as ANU92-197, the sample where putative biogenic graphite was found (*2*); SM/GR/97/5; and AK-98], and some magnetite-bearing rocks from Innersuartuut [171770 (*8*), 171771, and SM/GR/97/9]. For some samples (G91-26 and SM/GR/97/5), two fractions of powdered material were prepared from different splits of the coarsely crushed samples. All bulk measurements were duplicated, and all replicate analyses (different splits or different dissolutions) agreed within uncertainties. The bulk samples from Isua, Akilia, and Innersuartuut were found to have isotopically heavy Fe relative to igneous rocks (Fig. 1A).

Rocks from Akilia and Innersuartuut experienced granulite facies metamorphism in the early Archean (>750°C, 7 kbar) (*25*) and were retrogressed under amphibolite facies in the late Archean (*3*). Rocks from Isua experienced only amphibolite facies metamorphism (>550°C, 5 kbar) (*26*). The Fe isotopic compositions of pyroxene and magnetite mineral separates were similar to the compositions of the bulk rock samples from which they were extracted (within 0.08 ‰/amu) (Fig. 1B). Any original variations in F_{Fe} between the various minerals were probably obliterated by high-temperature equilibration during metamorphism. This finding is consistent with independent evidence for null-equilibrium isotope fractionation between magnetite and silicate minerals at high temperature (~790°C) (*27*).

Given the controversy regarding the possible igneous origin of the quartz-pyroxene rock on Akilia (*7–9*), one must consider whether the enrichment in heavy Fe is the result of metasomatic alteration of an original igneous protolith. The hydrothermal alteration of basalts can be thought of as a low pressure and temperature analog for such a metasomatic scenario. Altered mid-ocean ridge (MOR) basalts indeed have positive F_{Fe} values (*21*). However, such positive F_{Fe} values in these basaltic rocks correlate with a depletion in Fe concentration. If a similar loss of Fe occurred as a result of metasomatic alteration of the quartz-pyroxene rock on Akilia, it would be evident in the comparison of the ratios of Fe to an immobile trace element such as Ti between these samples and the surrounding igneous rocks. The high Fe/Ti ratios in the quartz-pyroxene rocks (Table 1 and Fig. 2) indicate that Fe was not preferentially lost. On the contrary, the high Fe/Ti ratios resemble those of BIFs found in the Isua Supracrustal Belt (*28*). A metasomatic origin (direct precipitation from a metamorphic fluid) can be ruled out as well, because fluid-rock interactions tend to enrich the fluids in the light isotopes (*14*, *21*), but no negative F_{Fe} values are observed in any of the quartz-pyroxene rock samples. Therefore, the enrichment in heavy Fe isotopes and high Fe/Ti ratios observed in the Akilia quartz-pyroxene rocks are most likely primary BIF signatures.

The detailed study of BIFs from the Kaapvaal Craton (*24*) provides a useful framework to interpret the positive F_{Fe} of BIFs from southwestern (SW) Greenland (Fig. 1B). The source of heavy Fe in these rocks is probably Fe(III) formed by oxidation of Fe(II) emanating from hydrothermal vents. The main carrier of Fe in Akilia rocks

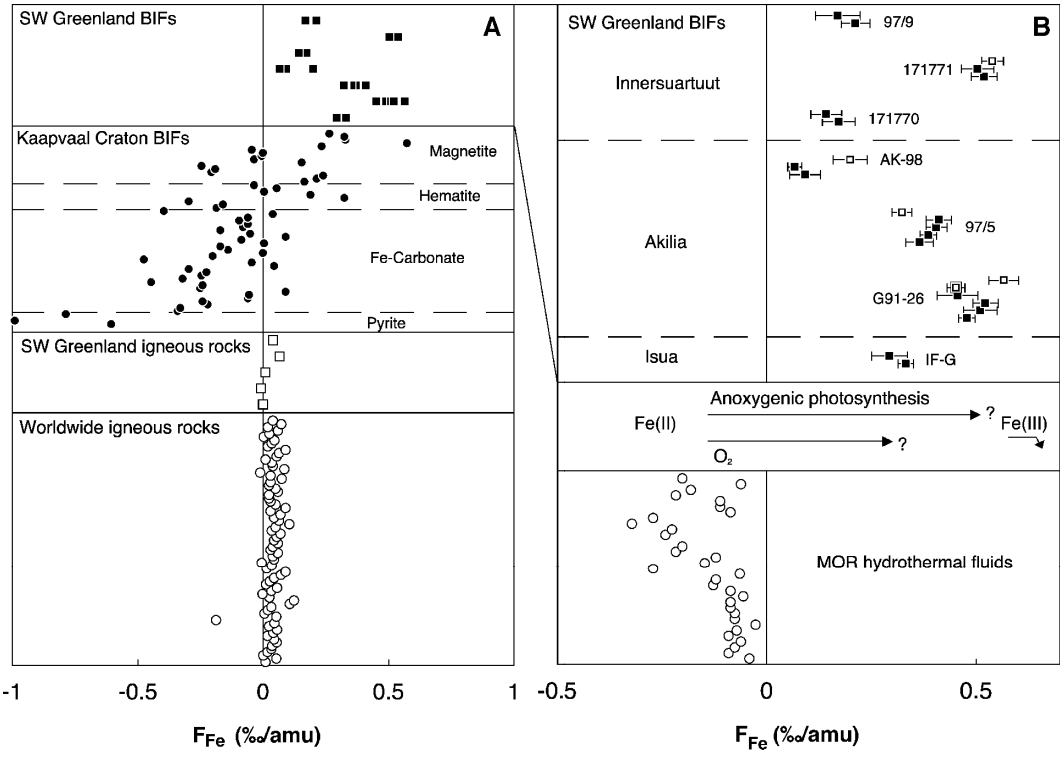

Fig. 1. F_{Fe} values (*13*) of worldwide igneous rocks (Table 1) (*12*, *15*, *20–23*), SW Greenland igneous rocks (Table 1), Kaapvaal Craton BIFs (*24*), SW Greenland banded rocks (Table 1), MOR hydrothermal fluids (*20*, *34*, *35*), and Fe(II) oxidation–Fe(III) precipitation experiments (*16–19*). (**A**) Worldwide (open circles) and SW Greenland (open squares) igneous rocks define a very homogeneous baseline. Kaapvaal Craton BIFs (solid circles) show a scatter in F_{Fe} with a strong mineralogical control. SW Greenland banded rocks (solid squares) are all enriched in heavy Fe relative to the igneous baseline. (**B**) MOR hydrothermal fluids (open circles) are enriched in light Fe. The experimental isotopic fractionation associated with oxidation of Fe(II) and subsequent precipitation of Fe(III) are indicated with arrows (note that the net effect is uncertain because of kinetic isotope fractionation during precipitation) (*17*, *19*). A closeup of SW Greenland banded rocks is shown (black squares are bulk samples, gray square is pyroxene, open squares are magnetite). The enrichment in heavy Fe of SW Greenland banded rocks may be caused by fractionation associated with oxidation and subsequent precipitation of Fe(II) from hydrothermal vents. Error bars are 95% confidence intervals.

Table 1. Fe isotopic compositions of meterorites, igneous rocks, and banded rocks from SW Greenland (*37*). Uncertainties are 2σ. qtz, quartz; mgt, magnetite; px, pyroxene.

Type	Sample	Description	Fe (mol g^{-1})	Fe/Ti	Fe(II)/Fe(III)	δ^{56}Fe (‰)	δ^{57}Fe (‰)	F_{Fe} (‰/amu)
Carbonaceous chondrites	Orgueil	CI1 (13.69 mg)	-	-	-	0.015 ± 0.074	0.108 ± 0.192	0.015 ± 0.032
	Murchison	CM2 (16.44 mg)	-	-	-	0.015 ± 0.096	0.049 ± 0.186	0.011 ± 0.038
	Allende	CV3.2 (9.96 mg)	-	-	-	0.040 ± 0.110	0.103 ± 0.083	0.031 ± 0.025
Worldwide igneous rocks	BCR-2	Basalt (Columbia River, WA)	-	-	-	0.054 ± 0.077	0.159 ± 0.212	0.033 ± 0.034
	DTS-2	Dunite (Twin Sisters, WA)	-	-	-	0.022 ± 0.041	-0.058 ± 0.072	-0.002 ± 0.016
	AGV-2	Andesite (Lake County, OR)	-	-	-	0.112 ± 0.081	0.165 ± 0.275	0.056 ± 0.037
	DD-5	Anhydrous lherzolite (Eifel, Germany)	-	-	-	0.025 ± 0.046	0.008 ± 0.231	0.012 ± 0.022
	T4D3#7	Basalt (Loihi, HI)	-	-	-	0.044 ± 0.060	0.111 ± 0.114	0.028 ± 0.024
	T4D3#3	Basalt (Loihi, HI)	-	-	-	0.082 ± 0.112	0.149 ± 0.187	0.045 ± 0.042
	T4D2#1	Basalt (Loihi, HI)	-	-	-	0.153 ± 0.111	0.138 ± 0.386	0.072 ± 0.051
	V5-40-56	Basalt (American-Antarctic ridge)	-	-	-	0.181 ± 0.059	0.266 ± 0.167	0.090 ± 0.026
SW Greenland igneous rocks	SM/GR/97/2	Granitoid gneiss (Akilia)	0.000649	10.80 ± 0.31	4.75	-0.002 ± 0.120	0.005 ± 0.174	0.000 ± 0.042
	SM/GR/97/3	Ultramafic gneiss (Akilia)	0.001805	23.25 ± 0.46	1.64	-0.030 ± 0.089	-0.018 ± 0.053	-0.007 ± 0.016
	SM/GR/97/4	Gabbroic rock (Akilia)	0.001138	27.55 ± 0.78	6.25	0.009 ± 0.035	0.083 ± 0.100	0.010 ± 0.015
	SM/GR/97/6	Ultramafic rock (Akilia)	0.001384	35.66 ± 0.71	4.20	0.114 ± 0.174	0.208 ± 0.102	0.068 ± 0.032
	SM/GR/97/7	Granitoid (Akilia)	0.001045	11.43 ± 0.32	3.99	0.081 ± 0.098	0.119 ± 0.125	0.040 ± 0.032
SW Greenland BIFs	IF-G	BIF (Isua)	0.006995	10,346 ± 1552	0.50	0.666 ± 0.038	0.976 ± 0.209	0.332 ± 0.018
						0.603 ± 0.119	0.856 ± 0.185	0.294 ± 0.043
	G91-26 #1	Banded qtz-px rock (Akilia)	0.001743	6,962.2 ± 1044.3	2.31	0.970 ± 0.061	1.416 ± 0.077	0.477 ± 0.020
						1.025 ± 0.097	1.516 ± 0.214	0.510 ± 0.040
	G91-26 #2	Banded qtz-px rock (Akilia)	0.001743	6,962.2 ± 1044.3	2.31	1.057 ± 0.083	1.546 ± 0.130	0.522 ± 0.030
						0.905 ± 0.113	1.391 ± 0.276	0.456 ± 0.048
	G91-26 px	Mineral separate (Akilia)	-	-	-	0.937 ± 0.052	1.242 ± 0.118	0.452 ± 0.022
	G91-26 mgt	Mineral separate (Akilia)	-	-	-	1.133 ± 0.079	1.686 ± 0.237	0.566 ± 0.035
	SM/GR/97/5	Banded qtz-px rock (Akilia)	0.001179	4,706 ± 712	10.98	0.732 ± 0.089	1.089 ± 0.149	0.365 ± 0.033
						0.770 ± 0.052	1.167 ± 0.091	0.387 ± 0.020
	SM/GR/97/5 II	Banded qtz-px rock (Akilia)	0.001179	4,706 ± 712	10.98	0.812 ± 0.053	1.206 ± 0.225	0.406 ± 0.025
						0.817 ± 0.069	1.259 ± 0.192	0.411 ± 0.030
	SM/GR/97/5 mgt	Mineral separate (Akilia)	-	-	-	0.648 ± 0.097	0.970 ± 0.080	0.323 ± 0.023
	AK-98	Banded qtz-px rock (Akilia)	0.001182	4,721 ± 714	9.07	0.199 ± 0.111	0.259 ± 0.149	0.092 ± 0.037
						0.131 ± 0.041	0.215 ± 0.090	0.067 ± 0.017
	AK-98 mgt	Mineral separate (Akilia)	-	-	-	0.410 ± 0.207	0.596 ± 0.133	0.200 ± 0.041
	171770	Banded qtz-px rock (Innersuartuut)	0.002117	367.5 ± 36.8	18.60	0.345 ± 0.108	0.517 ± 0.171	0.172 ± 0.039
						0.310 ± 0.097	0.379 ± 0.168	0.143 ± 0.037
	171771	Banded qtz-px-mgt rock (Innersuartuut)	0.006327	361.0 ± 10.8	1.59	1.052 ± 0.095	1.543 ± 0.118	0.519 ± 0.030
						1.034 ± 0.110	1.473 ± 0.162	0.504 ± 0.039
	171771 mgt	Mineral separate (Innersuartuut)	-	-	-	1.073 ± 0.059	1.640 ± 0.170	0.539 ± 0.026
	SM/GR/97/9	Banded qtz-px rock (Innersuartuut)	0.001353	720.2 ± 72.0	15.00	0.430 ± 0.092	0.628 ± 0.156	0.213 ± 0.034
						0.360 ± 0.126	0.437 ± 0.298	0.170 ± 0.053

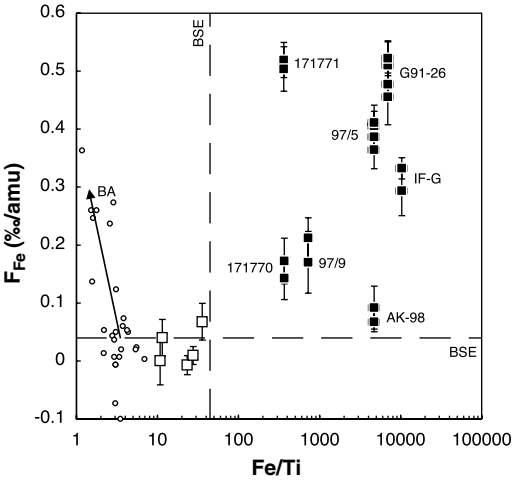

Fig. 2. Comparison between F_{Fe} (*13*) and the atomic ratio of Fe to Ti for igneous (open squares) and banded rocks (solid squares) from SW Greenland (Table 1). BSE indicates the composition of the bulk silicate Earth (*12*, *15*, *20–23*, *36*). Because Ti is an immobile element, it is a useful proxy for constraining the possible mobilization and transport of Fe. Basalt alteration (BA, open circles) (*21*) leads to a net loss of Fe and a correlated enrichment in heavy Fe isotopes. Banded rocks from Isua, Akilia, and Innersuartuut have high F_{Fe} and Fe/Ti relative to the surrounding igneous rocks. The high F_{Fe} may be caused by isotopic fractionation associated with Fe(II) oxidation (Fig. 1). The high Fe/Ti is because of the low solubility of Ti in the fluids that precipitated the BIFs. For Akilia samples to be derived by metasomatism from an igneous protolith, it would require that Fe be enriched by a factor of about 100 to 700 relative to the original formation. This is extremely unlikely and would not explain the associated enrichment in heavy Fe isotopes.

is pyroxene and not magnetite, as indicated by the high Fe(II)/Fe(III) ratios of the bulk rocks (Table 1). There is thus an apparent discrepancy between the heavy isotopic composition and the reduced speciation of Fe in these rocks. It is well established that all supracrustal rocks in Isua have been influenced by carbonate metasomatism (*29*), and as a result, BIFs have incorporated dolomite and calcite. Progressive granulite-facies metamorphism under low oxygen fugacity of such mineral assemblages would lead to pyroxene formation. This may explain the survival of positive Fe isotope signatures in pyroxene-dominated samples.

Various mechanisms have been proposed to describe the oxidation and subsequent precipitation of ferric Fe. These include abiotic photo-oxidation (*30*), direct oxidation by Fe(II)-oxidizing anoxygenic photoautotrophic bacteria (*24*, *31*), and O_2-mediated indirect oxidation by oxygenic photosynthetic bacteria (*32*). The isotopic fractionation associated with abiotic photo-oxidation has not yet been documented, but the latter two possibilities can be tested with Fe isotopes. Whether the oxidation is direct [Fe(II)-oxidizers] or indirect (O_2-mediated), the isotopic fractionation associated with hydrous ferric oxide (HFO) precipitation can be divided into two steps (*17*, *19*). The first step involves oxidation of $Fe(II)_{aq}$ into $Fe(III)_{aq}$ and is associated with an enrichment in the heavy isotopes. The second step involves precipitation of HFO and is probably associated not with equilibrium fractionation, but with a kinetic effect that enriches the precipitate in the light isotopes and depends on the rate of precipitation. The net isotope shifts documented for oxidation of Fe(II) and precipitation of HFO are +0.75 ‰/amu for anoxygenic photosynthetic oxidation (*16*) and +0.45 ‰/amu for O_2-mediated oxidation (*18*) under specific experimental conditions. If applied to hydrothermal fluids, the composition of the first precipitate (F_{Fe} around 0.3 to 0.6 ‰/amu) would agree for both mechanisms with the range of compositions measured in early Archean BIFs from SW Greenland.

Anoxygenic photosynthesis emerged on Earth before oxygenic photosynthesis (*33*). The measured Fe isotopic compositions in >3.83 Ga BIFs from SW Greenland are compatible with oxidation of Fe(II) by anoxygenic photoautotrophic bacteria. However, the Fe isotope fractionation in these rocks cannot necessarily be taken as a biosignature, because abiotic photo-oxidation can potentially cause a similar effect. More work is required to characterize Fe isotopic fractionation associated with photo-oxidation (*30*).

Iron isotopes, although not unambiguous biomarkers, do provide crucial information for revealing the protoliths of highly metamorphosed early Archean rocks. In the case of the quartz-pyroxene rock on Akilia Island, its enrichment in the heavy isotopes of Fe supports a sedimentary rather than an igneous origin. This conclusion agrees with the detection of S isotope anomalies in some of these rocks (*10*). The recognition of a sedimentary protolith in the earliest rock record can be important for studies of early life and ancient terrestrial environments, because ancient sediments represent the only direct record of early ocean chemistry.

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38. We thank A. Lepland, R. N. Clayton, F. M. Richter, O. Rouxel, C. N. Foley, R. Yokochi, and M. R. Savina for discussions; S. Moorbath and R. Yokochi for samples; R. Hines for technical assistance; L. Marin, J. Carignan, and the Service d'Analyze des Roches et des Minéraux for major and trace element analyses; and two anonymous reviewers for constructive comments. Supported by a Marie Curie Individual Fellowship from the European Union (M.v.Z.); by NASA grants NAG5-12997 (A.M.D.), NAG5-12077 (M.W.), and NAG5-7196 (M.W.); and by NSF grant EAR-9871154 (M.W.).

Supporting Online Material

www.sciencemag.org/cgi/content/full/306/5704/2077/DC1
Materials and Methods
Table S1
References

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Darwinian Selection on a Selfing Locus

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The shift to self-pollination is one of the most prevalent evolutionary transitions in flowering plants. In the selfing plant *Arabidopsis thaliana*, pseudogenes at the *SCR* and *SRK* self-incompatibility loci are believed to underlie the evolution of self-fertilization. Positive directional selection has driven the evolutionary fixation of pseudogene alleles of *SCR*, leading to substantially reduced nucleotide variation. Coalescent simulations indicate that this adaptive event may have occurred very recently and is possibly associated with the post-Pleistocene expansion of *A. thaliana* from glacial refugia. This suggests that ancillary morphological innovations associated with self-pollination can evolve rapidly after the inactivation of the self-incompatibility response.

The shift from outcrossing to self-fertilization (selfing) is classically regarded as one of the most prevalent evolutionary transitions in flowering plants (*1*). The extent of selfing and outcrossing can have profound effects on the levels and partitioning of genetic diversity in plant populations, the persistence of deleterious genetic polymorphisms, the allocation of resources within plants, and the diversification of floral morphology (*2–4*). Charles Darwin proposed the earliest model for the evolution of self-fertilization, the reproductive assurance model, which suggests selfing in plants can be evolutionarily advantageous when pollinators or mates are scarce in spite of inbreeding depression (*5*, *6*). Darwin's model also underlies Baker's Rule, which notes that colonizing species that disperse over long distances are generally self-compatible (*7*).

Arabidopsis thaliana is a predominantly self-pollinating plant with an outcrossing rate estimated at ~1% (*8*). In the Brassicaceae, the sporophytic self-incompatibility system enforces outcrossing by preventing pollen from germinating and developing on the stigma of a pistil from the same plant. Inactivation of at least one of the components of this system was a necessary step in the evolution of selfing in *A. thaliana*, because an active self-incompatibility response would prevent efficient self-pollination. The self-incompatible recognition system in the Brassicaceae is controlled by the *S* (*Sterility*) locus, which comprises a gene complex containing at least two functional genes. The *SRK/Aly13* gene encodes a transmembrane Ser/Thr receptor kinase expressed in the stigma, whereas the *SCR/SP11* gene encodes a small Cys-rich protein found in pollen coats that acts as a ligand to the SRK receptor protein (*9*, *10*). Studies have shown that both *SRK* and *SCR* are highly polymorphic, with allelic lineages maintained trans-specifically in several species in the Brassicaceae (*11–13*). Specific interactions between *SCR* and *SRK* alleles results in frequency-dependent selection that maintains a large number of alleles, as well as suppressed recombination to ensure the integrity of specific allelic interactions.

Both *SRK* and *SCR* have been shown to be pseudogenes in *A. thaliana*, located in an ~10-kb region of chromosome IV (*14*). The pseudogene *SRK* (Ψ*SRK*) in *A. thaliana* is expressed in stigmas but has been reported to contain a premature stop codon (*14*). Three distinct *A. thaliana SCR*-like pseudogenes linked to Ψ*SRK* have also been identified in *A. thaliana*. Ψ*SCR1* is located ~700 base pairs (bp) upstream of Ψ*SRK* and encodes a truncated open reading frame without three of eight conserved Cys residues believed to be required for the structural integrity of the SCR protein (*14*, *15*). Ψ*SCR2* and Ψ*SCR3* are located ~22 bp apart and ~8.5 kb upstream of Ψ*SRK* (*16*). These two alleles are highly truncated, do not encode long open reading frames, and share only patches of sequence similarity with the *SCR* signal sequence and 5′ untranslated region; given the very close proximity of these two pseudogenes, they are referred to together as Ψ*SCR2/3* (*14*). A recent transgenic study using the *A. lyrata SRK* and *SCR* genes demonstrates that both are necessary and sufficient for reestablishing the self-incompatible response in selfing *A. thaliana* and that the rest of the genes required to express the pollen rejection response remain largely intact in this species (*16*). This key result indicates that the Ψ*SCR* and/or Ψ*SRK* pseudogenes represent a selfing locus in *A. thaliana*, permitting self-fertilization to evolve sometime in the ~5 to 6 million years since the divergence of this species from *A. lyrata* (*17*).

We sequenced alleles of Ψ*SCR1* in 21 *A. thaliana* ecotypes across the Eurasian range of this species (*18*). Only four nucleotide polymorphisms were observed across 881 silent sites. The level of silent-site nucleotide diversity (π) for this pseudogene was 0.0012 (Fig. 1 and Table 1), which is one-sixth the mean π of 0.007 for *A. thaliana* nuclear genes (*19*). In contrast, *SP11/SCR* in the self-incompatible species *Brassica oleracea* is highly polymorphic, with silent-site nucleotide diversity π equal to 0.321 (*11*). In *A. lyrata*, the sister species to *A. thaliana*, two alleles of *SCR* are known and also display high levels of nucleotide divergence (*14*). The elevated levels of nucleotide polymorphism in related self-incompatible species are consistent with the action of frequency-dependent

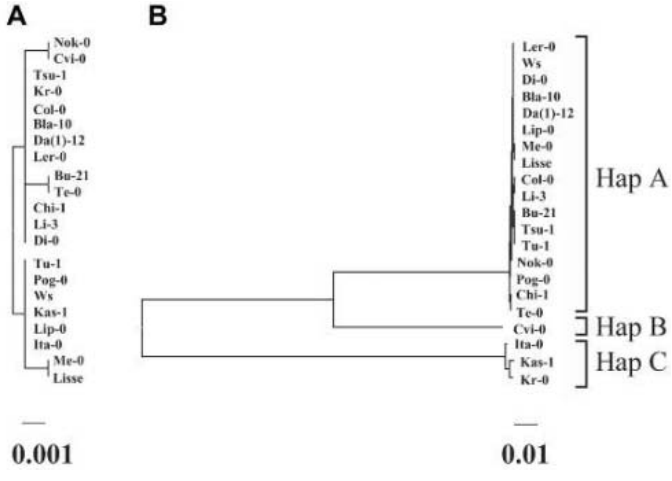

Fig. 1. Gene genealogy of *A. thaliana* (**A**) Ψ*SCR1* and (**B**) Ψ*SRK* alleles. The three different Ψ*SRK* haplotype groups are indicated as Hap A, Hap B, and Hap C. The scale for the branch lengths of the Ψ*SRK* genealogy is an order of magnitude greater than that for Ψ*SCR1*.

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selection acting on this gene in these outcrossing taxa, whereas the low level of nucleotide variation in Ψ*SCR1* suggests that the genomic region may have been the target of positive directional selection associated with the transition to selfing in *A. thaliana*.

We calculated the joint likelihood of the time since the completion of a putative selective sweep (T in $2N_e$ generations, where N_e is the effective population size) and the selection coefficient ($\beta = 4N_e s$, where s is a selection parameter) for the Ψ*SCR1* locus by simulating coalescent genealogies consistent with the mutational pattern found at the pseudogene (*18*, *20*). The joint likelihood surface (Fig. 2) demonstrates that the level of variation at Ψ*SCR1* is most consistent with a very recent selection event ($T \sim 0$) and a selection coefficient β greater than 20. We can use a likelihood ratio test to determine whether variation at Ψ*SCR1* is consistent with neutrality by comparing the likelihood of $\beta = 0$ maximized over T to that of the likelihood maximized over β and T. The first quantity corresponds to the null hypothesis that the pseudogene mutation is neutral, whereas the second corresponds to the alternative hypothesis that the mutation is nonneutral. The analysis indicates that we can reject a model in which a neutral mutation reaching fixation explains the low levels of variation at Ψ*SCR1* (likelihood ratio test statistic = 5.42, $P < 0.01$ using χ^2 approximation, $P < 0.02$ using simulations).

These results are consistent with recent directional selection acting on the Ψ*SCR1* pseudogene in *A. thaliana*. It is possible, however, that positive selection may be acting on one of the other closely linked self-incompatibility pseudogenes and that the observed effects on Ψ*SCR1* arise from genetic hitchhiking (*21*). In order to examine this possibility, we determined allelic variation at both Ψ*SCR2/3* and Ψ*SRK*.

The Ψ*SCR2/3* locus is located ~8.5 kb upstream of Ψ*SCR1* (*14*). Alleles of this pseudogene have nine single nucleotide polymorphisms across 823 silent sites in our sample of ecotypes, with silent-site π for this pseudogene equal to 0.0024 (Table 1). Although this region also has low levels of nucleotide variation, a likelihood ratio test cannot reject neutrality for Ψ*SCR2/3* ($P < 0.1$).

The *SRK* self-incompatibility pseudogene is located immediately downstream of Ψ*SCR1* in the *A. thaliana* Col-0 ecotype (Table 1 and Fig. 3) (*14*). Three distinct allele lineages or haplogroups of Ψ*SRK* were identified (Fig. 1), and the total nucleotide diversity estimate π was 0.078, whereas the synonymous-site π was 0.138. Members from all haplogroup classes remained transcriptionally active (*18*) despite the presence of disruptive mutations in most, but not all, alleles (Fig. 4). Although the three Ψ*SRK* haplogroups are highly divergent, several lines of evidence demonstrate that they are all located in the same physical position in the genome and are thus allelic to each other (supporting online text).

Variation at Ψ*SRK* has been affected by directional selection at Ψ*SCR1*. The nucleotide diversity level at Ψ*SRK* was higher than at the neighboring Ψ*SCR1* but was reduced relative to the ancestral diversity still observed in the sister species *A. lyrata*. Synonymous-site nucleotide diversity at Ψ*SRK* ($\pi = 0.138$) has been reduced to ~38% of that observed within *A. lyrata* ($\pi = 0.36$) (*13*). As many as 10 different haplotypes were observed in a global sampling of *A. lyrata* (*13*), whereas we found only three *A. thaliana* Ψ*SRK* haplogroups. Furthermore, one haplogroup (haplogroup A) consisting of nearly identical haplotypes predominated, with a frequency of 81% among the sequenced alleles (Fig. 1). This level and pattern of polymorphism is consistent with incomplete hitchhiking of Ψ*SRK* to the Ψ*SCR1* sweep (*22*, *23*) and also suggests that recombination must be present in the *A. thaliana* pseudo–*S* region.

Although recombination suppression has been observed in the *S* alleles of *A. lyrata SCR1* and *SRK*, parameteric and nonparametric methods indicate that significant levels of recombination are present on the *A. thaliana* pseudo–*S* region (*24*) (supporting online text and table S2). We estimated the population recombination rate $\rho = 2N_e r$, where r is the recombination rate, for the genomic region encompassing the *A. thaliana* self-incompatibility pseudogenes and the flanking *ARK3* and U-box protein-encoding gene. The estimate of ρ for the entire surveyed region is 16 and is significantly different from zero ($P < 0.001$). This is a conservative estimate, because the ancestral linkage disequilibrium among ancient polymorphisms still segregates, in part, within Ψ*SRK* (but not between the Ψ*SCR1* and Ψ*SRK* alleles). Estimates of recombination with Ψ*SCR1* and Ψ*SRK* combined were also calculated to evaluate whether recombination could decouple variation between these two pseudogenes (table S2); the estimate is still significantly different from zero ($P < 0.02$). Linkage disequilibrium is no longer required to maintain allelic interactions between Ψ*SCR1* and Ψ*SRK*, and it appears that recombination has evolved after the origin but before the global fixation of the pseudogene allele at *SCR*, resulting in differences in the evolutionary histories we observe among genes in this region.

These results indicate that the transition to selfing in *A. thaliana* arose as a consequence of positive selection on a pseudogene allele of *SCR* and not at *SRK*. The levels of nucleotide variation at this and other genes in and around

Table 1. Variation at the pseudo–self-incompatibility genomic region. Position of the genes along the chromosome IV sequence (GenBank accession no. NC_003075) is given in Mb; alignment length is the length of the sequenced region; π represents silent-site estimates of nucleotide diversity.

Gene	Position	Alignment length (bp)	Number of silent sites	π
U-box gene	11.3562–11.3573	617	161*	0.0606
Ψ*SCR2/3*	11.3753–11.3754	854	823†	0.0024
Ψ*SCR1*	11.3822–11.3831	883	881†	0.0012
Ψ*SRK*	11.3839–11.3871	2003	444*	0.1382
ARK3	11.3889–11.3932	834	384	0.0316

*Intron sites are either unalignable (Ψ*SRK*) or not present (the U-box gene), and only exon regions were analyzed. Moreover, Ψ*SRK* is expressed, and we excluded putative nonsynonymous sites. †Because these are pseudogenes, all sites are considered silent, excluding gaps.

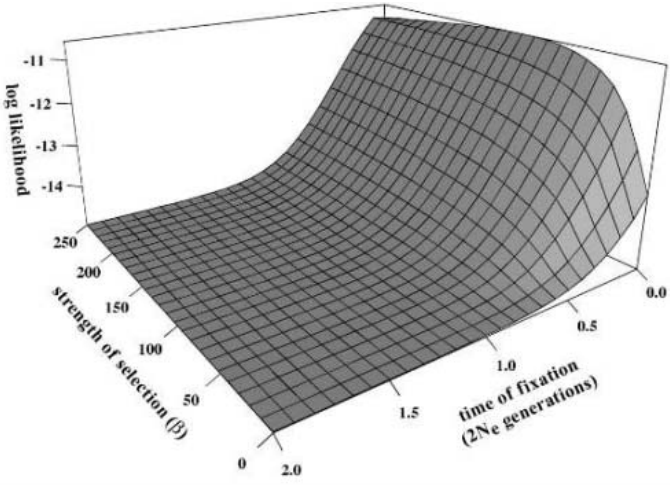

Fig. 2. The joint likelihood surface of the time since the end of the selective sweep ($T = 2N_e$) and the strength of selection ($\beta = 4N_e s$).

the *A. thaliana* pseudo–self-incompatibility region allow us to define the physical limits of selection in the genome. The higher level of nucleotide variation at Ψ*SRK* indicates that the selective sweep at Ψ*SCR1* is bounded at the 3′ end by the intergenic region between these two pseudogenes. This is confirmed by analysis of the *ARK3* kinase gene located ~2 kb downstream of the Ψ*SRK* sequence (Fig. 3) (*14*) and of the U-box protein-encoding gene located ~34 kb upstream of Ψ*SCR1* (Fig. 3) (*14*); both have elevated levels of nucleotide variation (Table 1). If we assume the distances between genes as determined in the Col-0 *A. thaliana* sequence, the adaptive sweep at Ψ*SCR1* affects a large genomic region between ~10 and ~35 kb in length.

The selective sweep associated with the fixation of the Ψ*SCR1* selfing allele appears to have occurred very recently. The 95% confidence intervals of time since the adaptive sweep at the Ψ*SCR1* selfing locus spans 0 to ~0.32 million years ago. In this period, glacial-interglacial climate changes occurred in 100,000-year cycles (*25*), and plant and animal species experienced expansions and contractions of their distributions. *A. thaliana* is thought to have experienced this typical pattern of geographic range distribution in Eurasia, through population expansion by colonization from refugia after glacial retreats (*26*, *27*). The statistical estimate of the timing of the transition to selfing (*T* maximal at 0) is compatible with a model of post-glacial expansion of *A. thaliana* ~17,000 years ago, when this species is thought to have expanded from Mediterranean and Central Asian refugia after the Pleistocene (*26*).

The recent origin of selfing in a time scale coincident with recent post-Pleistocene expansion suggests that self-pollination in *A. thaliana* evolved in line with Darwin's reproductive assurance model (*5*), because such an expansion in species range would presumably be accompanied by scarcities in outcrossing mates. These findings also provide the molecular underpinnings for the adherence of this species to Baker's Rule (*7*), because the evolution of selfing facilitates the ability of this species to colonize habitats over long distances. This is the first demonstration that the molecular evolution of selfing alleles is driven by positive directional selection.

The evolution of self-fertilization in plants is associated with several physiological and morphological changes, including the relative position and timing of maturation of the stamens and stigma (*3*). For example, flowers of *A. thaliana* are the smallest in the genus (petal length, 3 to 4 mm), a feature which reduces the costs associated with outcrossing, compared to all other sister species in the genus (4 to 10 mm) (*28*). It is likely, however, that the inactivation of the self-incompatibility genes represent the first step in the evolution of selfing in *A. thaliana*, because any changes in floral morphology that promote self-pollination will be deleterious if plants remain self-incompatible. Our results indicate that subsequent adaptations in floral morphology correlated with the evolution of self-pollination can quickly evolve after the loss of self-incompatibility, allowing for the rapid establishment of the selfing syndrome in colonizing plant species. Indeed, at least one inflorescence developmental gene, *TFL1*, shows evidence of a recent selective sweep (*29*), and analysis of other genes underlying morphological and physiological correlates of selfing should also reveal signatures of recent directional selection. These findings support the contention that adaptations such as those associated with key mating system innovations can occur very rapidly and allow species to exploit new habitats.

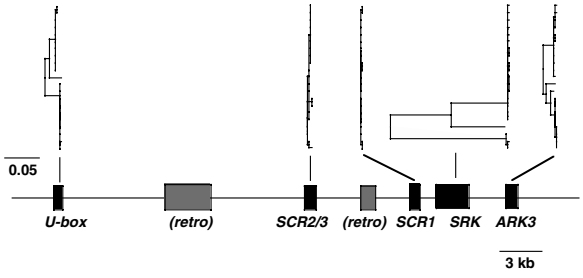

Fig. 3. Genealogies for genes across the pseudo–*S* locus of *A. thaliana*. Reduced nucleotide variation at the Ψ*SCR1* and Ψ*SCR2/3* allele trees results in short branch lengths, whereas the other three genes in the region have long internal branches. Positions of the genes are depicted according to the chromosome IV sequence (GenBank accession no. NC_003075). *Retro* denotes sequence with homology to a *copia*-like retrotransposon (left retrotransposon, At4g21360; right retrotransposon, At4g21363).

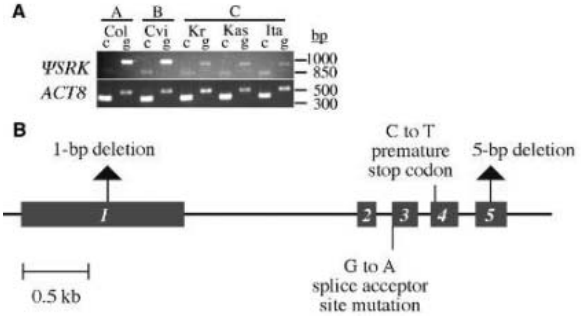

Fig. 4. Expression and disruptive mutations of the Ψ*SRK* gene. (**A**) Reverse transcription polymerase chain reaction analysis of Ψ*SRK* for ecotypes containing haplogroup A (Col-0), B (Cvi-0), and C (Kr-0, Kas-1, and Ita-0) alleles. The *ACT8* gene was amplified as a control. Both cDNA (c) and genomic DNA (g) were used for the amplification. (**B**) The haplogroup A and B mutations are shown above and below the Ψ*SRK* gene diagram, respectively. Examination of the Ψ*SRK* gene sequence from 21 ecotypes revealed multiple, independent gene-disruptive mutations in most alleles. The exon 4 premature stop mutation previously identified in Col-0 was found in 13 of the 17 haplogroup A alleles but not in the remaining four haplogroup A alleles or in haplogroups B or C. This indicates that the previously identified stop codon mutation (*14*) is not solely responsible for the evolutionary transition to selfing in *A. thaliana*. Three of the 13 haplogroup A alleles that have the exon 4 stop codon mutation also have an additional 1-bp frameshift deletion in exon 1. Of the four haplogroup A alleles that do not contain the stop mutation in exon 4, two possess a 5-bp frameshift deletion in exon 5 that alters the sequence of the encoded kinase region. The Cvi-0 ecotype (haplogroup B) has a splice site mutation at the end of intron 2, resulting in a frameshift. Analysis of the cDNA revealed no obvious inactivating mutation for ecotypes Ita-0, Kas-0, or Kr-0, all members of haplogroup C. The lengths of the exons and introns are depicted based on the Cvi-0 gene structure.

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Acetylation by Tip60 Is Required for Selective Histone Variant Exchange at DNA Lesions

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Phosphorylation of the human histone variant H2A.X and H2Av, its homolog in *Drosophila melanogaster*, occurs rapidly at sites of DNA double-strand breaks. Little is known about the function of this phosphorylation or its removal during DNA repair. Here, we demonstrate that the *Drosophila* Tip60 (dTip60) chromatin-remodeling complex acetylates nucleosomal phospho-H2Av and exchanges it with an unmodified H2Av. Both the histone acetyltransferase dTip60 as well as the adenosine triphosphatase Domino/p400 catalyze the exchange of phospho-H2Av. Thus, these data reveal a previously unknown mechanism for selective histone exchange that uses the concerted action of two distinct chromatin-remodeling enzymes within the same multiprotein complex.

DNA double-strand breaks (DSBs) are a deleterious type of DNA damage leading to chromosomal breakage. Cells have developed mechanisms to detect and repair DSBs (*1*, *2*), which must access nucleosomal DNA. Two classes of activities regulate the accessibility of DNA by either covalently modifying histones or using adenosine triphosphate (ATP) hydrolysis to catalyze histone mobilization (*3*, *4*). Current knowledge suggests that covalently modified histones can create specific interaction sites for regulatory proteins and complexes (*5*, *6*).

Incorporation of histone variants into nucleosomes provides another mechanism for altering chromatin structure (*7*). Whereas the major histones are assembled into nucleosomes during DNA replication, histone variants can be incorporated into chromatin in a replication-independent manner (*8*–*10*). An example of such an activity is the yeast Swr1p ATPase complex, which catalyzes the exchange of H2A for the variant H2A.Z in nucleosomes (*9*–*11*).

Histone modifications can mark distinct chromatin locations. H2A.X, an essential mammalian histone variant required for genomic stability, becomes phosphorylated at sites of DSBs by conserved DNA damage–recognizing factors (*12*, *13*). Like H2A.X, H2A and H2Av become phosphorylated at DSBs in yeast and flies, respectively (*14*, *15*). Because repair requires access to DNA, it has been suggested that this phosphorylation might attract chromatin-remodeling complexes to DSBs (*16*). The removal of phospho-H2A.X is replication-independent and could be catalyzed by the same complexes. DSBs accumulate upon inactivation of the human Tip60 complex, implicating it as one candidate for a chromatin-remodeling complex with a role in DNA repair (*17*).

We demonstrate that the *Drosophila* dTip60 multiprotein complex catalyzes exchange of phospho-H2Av with unmodified H2Av. This reaction is catalyzed by two chromatin-dependent enzymes within the dTip60 complex: the histone acetyltransferase dTip60 and the ATPase Domino. These factors sequentially acetylate and then replace nucleosomal phospho-H2Av with H2Av from within the dTip60 complex.

The dTip60 complex was purified from *Drosophila* S2 cells. dPontin, the fly homolog of a subunit of the human Tip60 complex (*17*), was epitope-tagged with a hemagglutin (HA)-Flag tag at the C terminus (fig. S1). The dPontinHAFlag-associated proteins were isolated from nuclear extracts by sequential Flag- and HA-affinity purification followed by a glycerol gradient (fig. S1). Peak fractions of dPontin-HAFlag, dTip60, and Domino were identified by immunoblotting (Fig. 1A) and assayed for histone acetyltransferase activity (fig. S2). Several polypeptides that copurified with dPontin-HAFlag (fig. S1) were identified by multidimensional protein identification technology

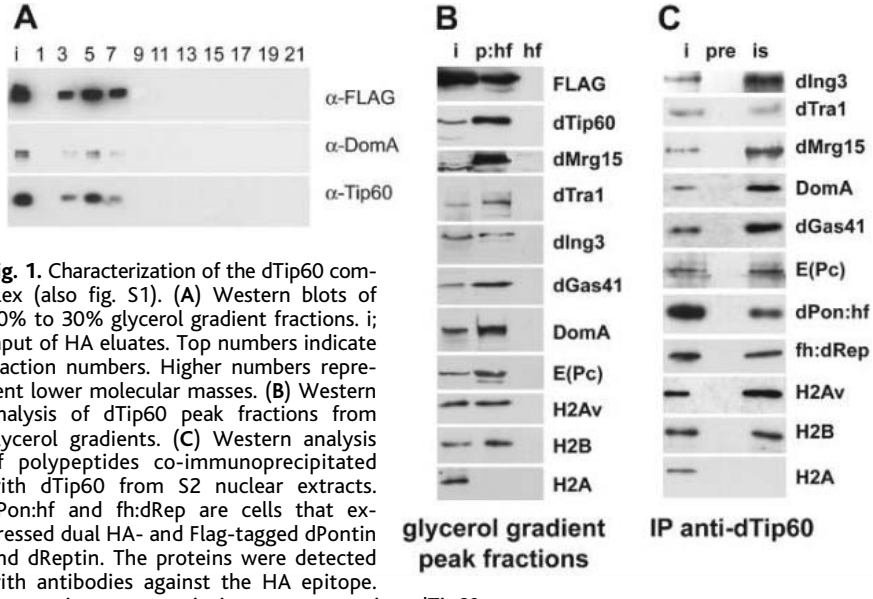

Fig. 1. Characterization of the dTip60 complex (also fig. S1). (**A**) Western blots of 10% to 30% glycerol gradient fractions. i; input of HA eluates. Top numbers indicate fraction numbers. Higher numbers represent lower molecular masses. (**B**) Western analysis of dTip60 peak fractions from glycerol gradients. (**C**) Western analysis of polypeptides co-immunoprecipitated with dTip60 from S2 nuclear extracts. dPon:hf and fh:dRep are cells that expressed dual HA- and Flag-tagged dPontin and dReptin. The proteins were detected with antibodies against the HA epitope. pre; pre-immunesera, is; immunesera against dTip60.

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(MudPIT) (*18*). We identified polypeptides with homology to all 16 subunits of the human Tip60 complex (table S3). This analysis also revealed a substantial number of tryptic peptides from histones H2Av and H2B but not from other histones.

We raised antibodies against dTip60, dMrg15, dTra1 (*19*), dGas41, dIng3, and E(Pc). These antisera and antibodies against Domino, H2Av, and H2B were used in immunoblotting of gradient peak fractions and anti-dTip60 immunoprecipitates from nuclear extracts to confirm that these proteins are part of the dTip60 complex (Fig. 1, B and C). dPontin-HAFlag stably associated with all dTip60 complex subunits examined, including dReptin, the fly homolog of the human Tip60 complex component Tip49b (Fig. 1C) (*17*). Histones H2Av and H2B stably associated with the dTip60 complex, whereas histone H2A and other histones were not detected (Fig. 1, B and C) (*20*).

Tip60 complexes function in DSB repair and contain the ATPase Domino/P400 and H2Av/H2B heterodimers. Because H2Av becomes phosphorylated at sites of DSBs (*15*), we tested whether dTip60 complex remodeled nucleosomes containing phospho-H2Av. We assembled recombinant *Drosophila* nucleosomes containing H2Av with a point mutation that mimicked phosphorylation at Ser^{137} (Ser^{137} to Glu^{137}; H2AvE) (Fig. 2A and fig. S4) (*21*). Upon incubation with the dTip60 complex, recombinant H2AvFlag/H2B heterodimers, acetyl-coenzyme A (acetyl-CoA), and ATP, a transfer of H2AvFlag to the nucleosomal arrays was observed (Fig. 2B, lanes 7 to 9, and figs. S5 and S6). The transfer reaction proceeded rapidly (notable amounts of H2AvFlag were incorporated within 5 min; lane 7) and depended on the presence of nucleosomes (no transfer onto free DNA occurred; lane 2). Although relatively small amounts of H2AvFlag were transferred in the absence of ATP and/or acetyl-CoA (lanes 4 to 6), it was about seven times more efficient in the presence of both cofactors (lane 9). Addition of a nonhydrolyzable ATP analog (γS-ATP; lane 10) reduced the background

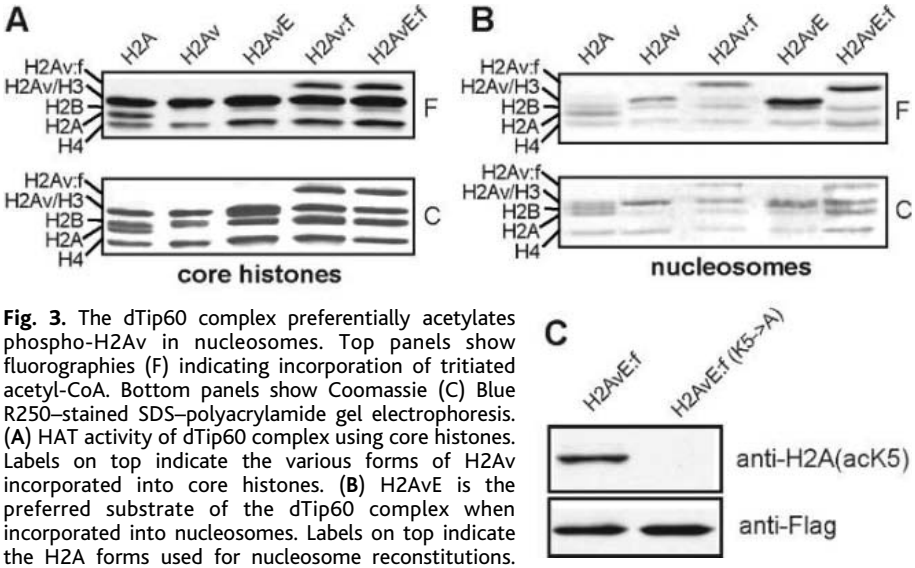

Fig. 3. The dTip60 complex preferentially acetylates phospho-H2Av in nucleosomes. Top panels show fluorographies (F) indicating incorporation of tritiated acetyl-CoA. Bottom panels show Coomassie (C) Blue R250–stained SDS–polyacrylamide gel electrophoresis. (**A**) HAT activity of dTip60 complex using core histones. Labels on top indicate the various forms of H2Av incorporated into core histones. (**B**) H2AvE is the preferred substrate of the dTip60 complex when incorporated into nucleosomes. Labels on top indicate the H2A forms used for nucleosome reconstitutions. Note that histone H4 is not the preferred substrate of Tip60-type complexes in recombinant nucleosomes. (**C**) Antibodies against H2A(acK5) recognize acetyl-K5 of H2AvE. Recombinant nucleosomal arrays were acetylated by the dTip60 complex. Antibodies against H2A(acK5) fail to recognize acetylated arrays containing H2AvE:f (K5->A). Membrane was probed with antibodies against Flag as loading control.

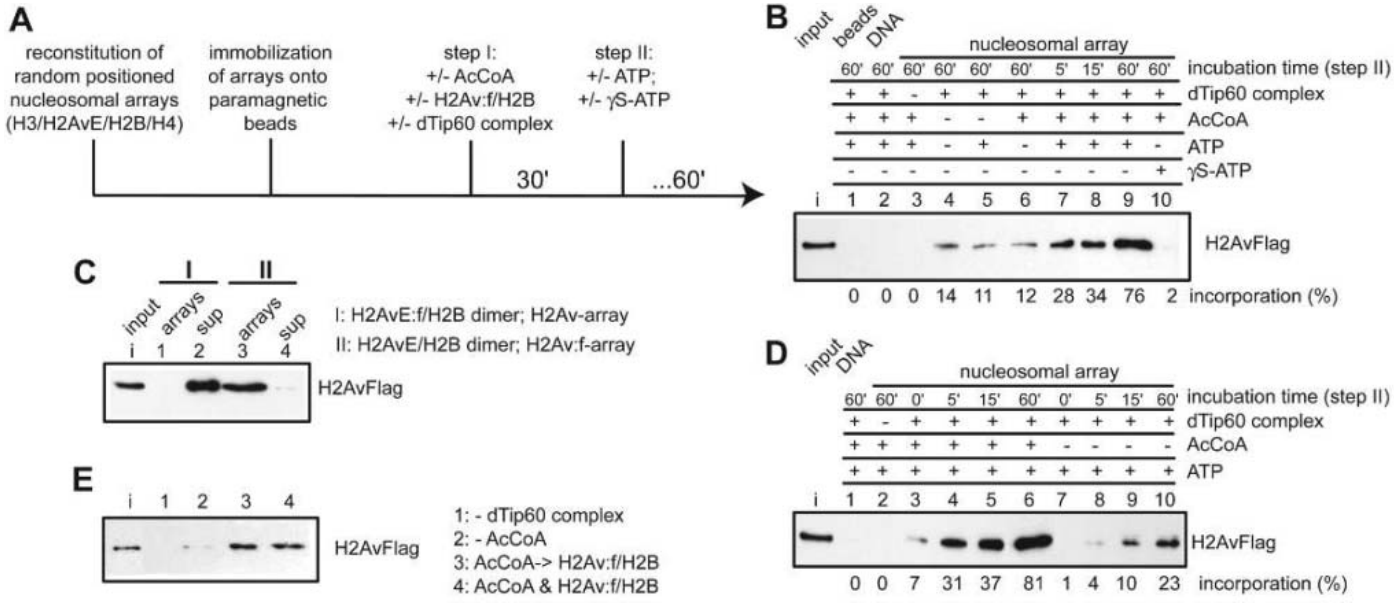

Fig. 2. The dTip60 complex exchanges phospho-H2Av from nucleosomal arrays with H2Av. (**A**) Experimental scheme. (**B**) Western analysis of nucleosomal arrays probed for incorporated H2AvFlag (H2Av:f) with antibodies against Flag. Input is 20% of the H2Av:f/H2B heterodimers used in each reaction. Added substrates and incubation times are indicated at the right. γS-ATP, adenosine-5′-O-(3-thiotriphosphate). (**C**) The dTip60 complex preferentially incorporates H2Av into phospho-H2Av-containing nucleosomes but not vice versa. Arrays, nucleosomal arrays; sup, supernatants. Roman numerals indicate the combinations of heterodimers and arrays used in the reaction as indicated to the right [the exposure shown is part of the same exposure and blot shown in (B)]. (**D**) Histone exchange by the dTip60 complex is enhanced by the presence of acetyl-CoA. For details, see (B). (**E**) The acetylation of nucleosomes is essential for optimal histone exchange. H2Av/H2B heterodimers were added either after preacetylating of nucleosomal arrays containing H2AvE for 30 min (lane 3) or simultaneously with AcCoA (lane 4). No significant difference in amounts of H2Av:f incorporation was observed.

activity of the complex. The dTip60 complex was highly selective for incorporation of H2Av into H2AvE-containing nucleosomal arrays (Fig. 2C). No H2AvEFlag was incorporated into nucleosomes containing H2Av (lanes 1 and 2), and no significant release of H2AvFlag was observed from nucleosomal arrays in the presence of H2AvEFlag/H2B heterodimers (lanes 3 and 4; also fig. S7). Time course experiments revealed that the presence of acetyl-CoA enhanced the transfer speed and the quantity of H2Av incorporation (Fig. 2D, compare lanes 6 and 10). The incorporation rate of H2AvFlag into the nucleosomal arrays was unchanged when acetyl-CoA only was temporarily added to the exchange reactions and removed before the addition of heterodimers (Fig. 2E, lanes 3 and 4). This strongly suggests that the acetylation of the nucleosomal arrays by the dTip60 complex, but not of heterodimers, is crucial for optimal H2Av exchange.

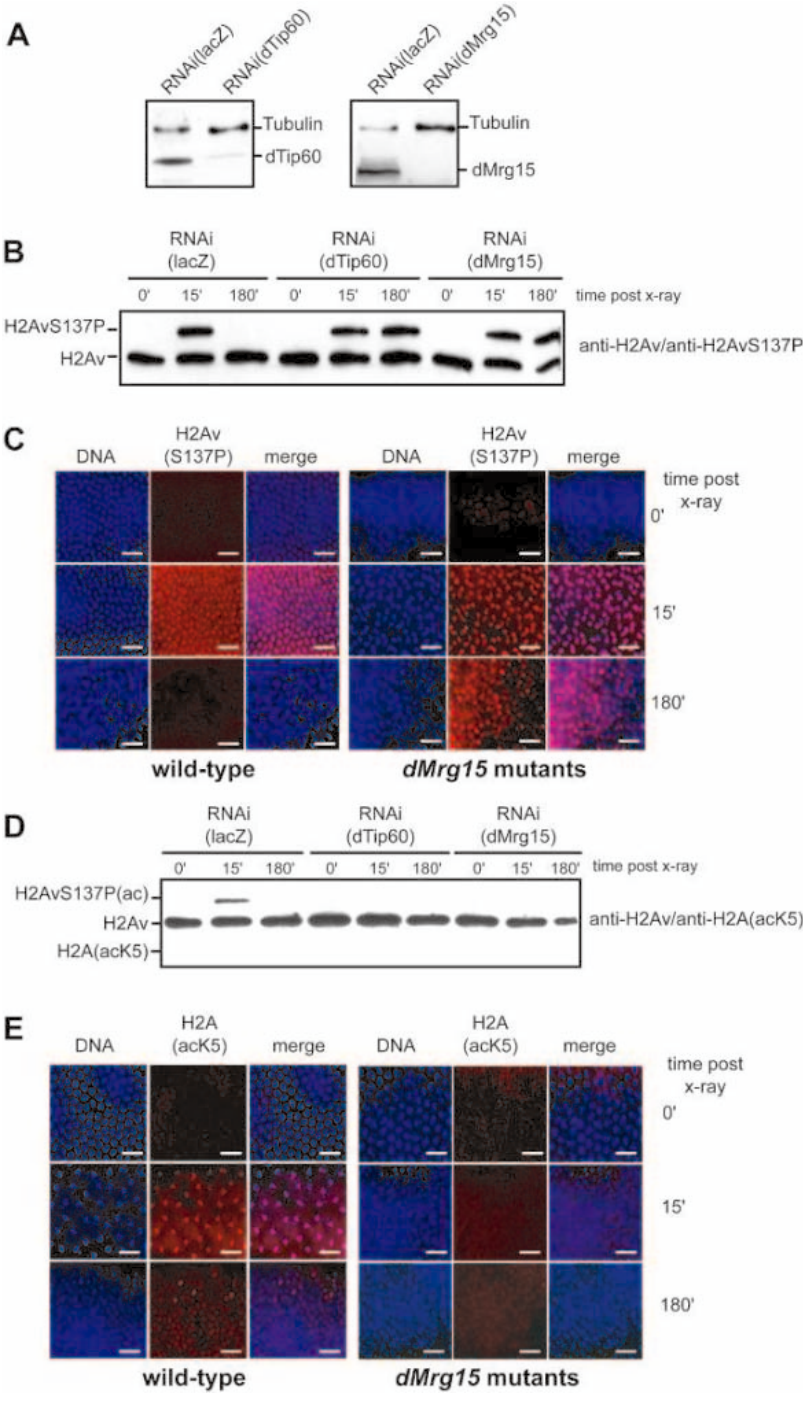

Fig. 4. Loss of dTip60 complex leads to the accumulation of phospho-H2Av upon DNA damage and abolishes DSB-dependent transient acetylation of phospho-H2Av. (**A**) RNAi assays for lacZ, dTip60, and dMrg15. Western blots of extracts from dsRNA-transfected cells. (**B**) Western blots of chromatin extracts from dsRNA-treated cells. After RNAi-treatment, the cells were γ-irradiated [50 gray (Gy)] and harvested at given time points. The blots were probed for H2Av and phospho-H2Av (H2AvS137P). (**C**) Close-up of whole-mount embryos immunolabeled for phospho-H2Av (red) 0, 15, and 180 min after γ irradiation. The DNA was counterstained with 4′,6′-diamidino-2-phenylindole (blue). Left images are wild-type embryos; right images are *dMrg15* mutants (also fig. S8). (**D**) Chromatin extracts of dsRNA-treated and γ-irradiated cells were probed with antibodies against H2A(acK5) and H2Av. (**E**) Magnification of whole-mount embryos stained with antibodies against H2A(acK5) (red; DNA in blue; also fig. S9). For details, see (C). White bars indicate 10 μm.

To examine the acetyltransferase specificity of the dTip60 complex, we used different combinations of recombinant histones as substrates in histone acetyltransferase (HAT) assays. In the presence of core histones, H2A, H2Av, and H2AvE were acetylated at equally low levels (Fig. 3A). However, in a nucleosomal context, acetylation of H2AvE was significantly increased over that observed for all other histones (Fig. 3B). This confirms that the dTip60 complex preferentially targets and acetylates phospho-H2Av in nucleosomes. In fact, Lys^5 of histone H2Av is acetylated by the dTip60 complex (Fig. 3C). As individual monomeric histones, H2A, but not H2Av or H2AvE, was the preferred substrate of the dTip60 complex (fig. S7). By contrast, acetylation was about equal between H2A and H2Av when heterodimers with H2B were assayed, whereas acetylation of H2AvE was unchanged (fig. S7). Thus, dTip60 complex prefers H2Av-containing heterodimers over those containing H2AvE.

Upon induction of DSBs, phospho-H2Av rapidly accumulates on chromatin with peak amounts after 10 to 15 min (*15*). During the course of DNA repair, this phosphorylation becomes undetectable within 180 min. The dTip60 complex acetylates and removes phospho-H2Av from nucleosomes in vitro. Thus, we tested whether removal of phospho-H2Av during repair was dependent on dTip60 complex in vivo. We depleted dTip60 or dMrg15 from S2 cells by RNA interference (RNAi) (Fig. 4A) (*18*). These cells were exposed to γ irradiation to induce DSBs, and the nucleosomal histones were extracted after 0, 15, and 180 min. The amounts of H2Av and phospho-H2Av were compared by immunoblotting (Fig. 4B). In mock-treated cells, phospho-H2Av levels peaked after 15 min and were undetectable after 180 min (Fig. 4B). By contrast, phospho-H2Av levels remained high in cells depleted for either dTip60 or dMrg15. To confirm these findings in embryos, we generated a null allele of *dMrg15* (*18*) and tested phospho-H2Av levels after γ irradiation. Again, the levels of phospho-H2Av remained higher in *dMrg15* mutants than in wild-type embryos (Fig. 4C and fig. S8).

Because the dTip60 complex acetylated nucleosomal phospho-H2Av in vitro, we tested dependence of H2Av acetylation on dTip60 complex components in vivo. We probed chromatin extracts from γ-irradiated double-stranded RNA (dsRNA)–treated S2 cells as well as *dMrg15* mutant embryos with antibodies against H2A(acK5), which recognized H2Av(acK5) (Fig. 3C). We detected transient acetylation of a protein band that exhibits the migratory properties of phospho-H2Av (Fig. 4D). This acetylation was most prominent 15 min after γ irradiation and was not detected in extracts of cells lacking dTip60 or dMrg15. Similar observations were made by immunolabeling *dMrg15* mutant embryos (Fig. 4E and fig. S9). We conclude that the dTip60 complex acetylates nucleosomal phospho-H2Av at Lys^5 in a DSB-dependent manner.

The *Drosophila* dTip60 complex is structurally homologous to its human counterpart (Fig. 1 and table S3) (*17*, *22*, *23*). Both complexes share factors that are linked to cancer, transcription, and DNA repair, including Pontin, Reptin, Mrg15, Tra1, E(Pc), Gas41, and Tip60. We also identified the histone variant H2Av within the *Drosophila* dTip60 complex. The human Tip60 complex is essential for DSB repair and regulation of apoptosis, two processes that have been linked to histone H2Av in flies (*15*, *17*). Also the yeast NuA4 complex appears to accumulate at DSBs (*24*).

We demonstrated that the *Drosophila* dTip60 complex acetylates nucleosomal phospho-H2Av and exchanges it with an unmodified H2Av (Figs. 2 and 3 and figs. S4 to S6). The histone-exchange reaction catalyzed by the ATPase Domino is enhanced by dTip60-mediated acetylation of nucleosomal phospho-H2Av. It appears likely that phospho-H2Av recruits the dTip60 complex to DSBs to facilitate chromatin remodeling during DNA repair. In yeast, the DNA damage–dependent H2A kinase Mec1 genetically interacts with subunits of the NuA4 complex (*21*, *25*), and cells missing NuA4 subunits are sensitive to DSB-inducing agents (*25*, *26*). The physiological roles of the dTip60-mediated phospho-H2Av removal at sites of DSBs could not be clearly separated from a potential function of this complex in DSB repair because of the intimate temporal link between DSB repair and phospho-H2Av clearance (*20*). However, the overexpression of phospho-H2Av did not induce G2/M arrest or affect DSB-dependent G2/M arrest (fig. S10) (*14*, *21*), suggesting that this signal is not sufficient for damage checkpoint control.

The loss of human Tip60 leads to the accumulation of DSBs and is linked to a growing number of cancer types (*26*, *27*). The histone variant H2A.X is essential for genomic stability and a candidate tumor suppressor (*13*, *28*, *29*). Thus, our findings help to understand the functional link between DNA damage–dependent H2A.X phosphorylation and the role of Tip60-type complexes during DSB repair in chromatin.

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Supporting Online Material

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Mammalian Tissue Oxygen Levels Modulate Iron-Regulatory Protein Activities in Vivo

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The iron-regulatory proteins (IRPs) posttranscriptionally regulate expression of transferrin receptor, ferritin, and other iron metabolism proteins. Although both IRPs can regulate expression of the same target genes, $IRP2^{-/-}$ mice significantly misregulate iron metabolism and develop neurodegeneration, whereas $IRP1^{-/-}$ mice are spared. We found that $IRP2^{-/-}$ cells misregulated iron metabolism when cultured in 3 to 6% oxygen, which is comparable to physiological tissue concentrations, but not in 21% oxygen, a concentration that activated IRP1 and allowed it to substitute for IRP2. Thus, IRP2 dominates regulation of mammalian iron homeostasis because it alone registers iron concentrations and modulates its RNA-binding activity at physiological oxygen tensions.

Iron-regulatory proteins 1 and 2 (IRP1 and IRP2) bind with high affinity to RNA motifs known as iron-responsive elements (IREs) in numerous transcripts related to iron homeostasis. In ferritin H and L chains (H, heart; L, liver), IRP binding near the 5′ end of the mRNA inhibits translation. In contrast, binding of IRPs to IREs in the 3′ portion of the transferrin receptor 1 (TfR1) mRNA stabilizes TfR transcripts (*1*–*3*). Although the genes encoding IRP1 and IRP2 are highly homologous, they sense cytosolic iron levels by different mechanisms. IRP1 is a bifunctional protein; in iron-replete cells, IRP1 contains a cubane [4Fe-4S] cluster and functions as a cytosolic aconitase, interconverting citrate and isocitrate, whereas when the Fe-S cluster is absent, IRP1 apoprotein binds IREs with high affinity (*4*). Unlike IRP1, IRP2 undergoes iron-dependent degradation in iron-replete cells (*5*, *6*). Thus, neither IRP binds to IREs when cells are iron-replete, and ferritin expression increases accordingly, while TfR expression decreases.

In cell lines derived from a wide variety of tissues, IRP1 generally appears to be the major contributor to total iron-regulatory activity (*1*–*3*). In primary cultures of wild-type

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(WT) mouse embryonic fibroblasts, IRE-binding activity of IRP1 increased markedly in iron-depleted cells and greatly exceeded that of IRP2 under both iron-replete and iron-depleted conditions (Fig. 1A). In addition, increased IRE-binding activity of IRP1 correlated with a marked decrease in cytosolic aconitase activity (Fig. 1B).

Despite the ability of IRP1 to significantly modulate its IRE-binding activity in cell culture according to iron status, and its ability to regulate expression of target transcripts (*7*, *8*), animals in which IRP1 is genetically ablated display normal regulation of iron metabolism in most tissues (*9*). In contrast, animals that lack IRP2 express abnormally high amounts of ferritin and low amounts of TfR in multiple tissues, and they develop progressive adult-onset neurodegeneration (*10*). In liver lysates from iron-deficient WT animals, the IRE-binding activity of IRP2 increased in response to iron deficiency, whereas the IRE-binding activity and aconitase activity of IRP1 remained constant (Fig. 1, C and D) (*9*).

To understand why the regulatory activities of IRP1 and IRP2 differed in animal tissues compared to cell lines, we analyzed primary cultures of macrophages and lymphocytes from WT, IRP1$^{-/-}$, and IRP2$^{-/-}$ animals. In both cell types, IRP1 appeared to be the predominant IRE-binding protein when cells were cultured in atmospheric oxygen (21% O_2). Because mammalian tissue oxygen concentrations are closer to 3 to 6% than 21% (*11*–*14*), and because IRP activities can be affected by oxygen (*15*), these experiments were carried out at oxygen concentrations ranging from 3 to 21% to evaluate how IRP activities vary accordingly. In macrophages, the change in oxygen concentration from 3 to 21% led to a dramatic switch in the proportion of IRE-binding activity contributed by each IRP. IRP2 was the predominant source of IRE-binding activity at 3% O_2, whereas IRP1 became the major source of IRE-binding activity at 21% O_2. As IRP1 activity increased at 21% O_2, IRP2 levels decreased. In addition, at 3% O_2, IRP1 lost most of its ability to respond to changes in iron status (Fig. 2A and fig. S1). Thus, the relative importance of each IRP in the regulation of iron metabolism varied greatly with changes in oxygen concentration.

In spleen-derived lymphocytes, IRE binding activity of IRP1 also increased markedly as the oxygen concentration rose from 3 to 21% (Fig. 2C). IRP2 levels were highly regulated by iron at all oxygen concentrations examined, although IRP2 protein levels decreased gradually as the oxygen concentration increased (Fig. 2D). Thus, oxygen exposure is a critical variable that determines the potential regulatory activity of IRP1 and IRP2 and their relative importance in both macrophages and lymphocytes.

To evaluate the effect of oxygen-dependent changes of IRP activity on regulated targets, we compared ferritin and TfR expression levels in WT, IRP1$^{-/-}$, and IRP2$^{-/-}$ primary cell cultures grown in 21 or 3 to 6% O_2 concentrations. WT macrophages decreased ferritin and increased TfR expression in response to iron deficiency at both low and high oxygen concentrations (Fig. 3, A and B). In contrast, iron-deficient macrophages from IRP2$^{-/-}$ animals could not repress ferritin or

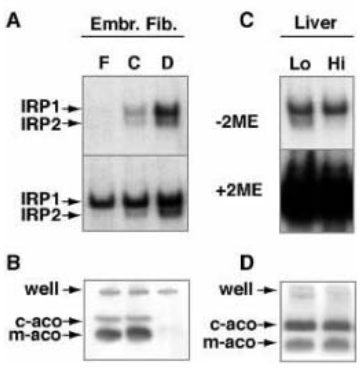

Fig. 1. IRP1 is an effective iron sensor in cultured mouse embryonic fibroblasts, but not in mouse liver. (**A**) Embryonic fibroblasts grown for 16 hours in 21% O_2 in unsupplemented medium (lane C), medium supplemented with 300 μM ferric ammonium citrate (FAC) (lane F), or 100 μM of the iron chelator deferoxamine mesilate (Dfo) (lane D) show increased IRE-binding activity of IRPs in a gel-shift assay in iron-depleted cells. IRP1 can be recruited from its non–IRE-binding form to bind IREs by addition of 2% β-mercaptoethanol (+2ME), which indirectly assesses total IRP1 levels. (**B**) IRP1 from iron-replete cells is an active aconitase (lanes F and C), but IRP1 in iron-depleted cells loses aconitase activity as IRP1 switches to the IRE-binding form (lane D). The mitochondrial aconitase (m-aco) and cytosolic aconitase (c-aco) bands were identified as described (*9*). (**C**) Liver lysates from WT animals on low- or high-iron diets show that IRE-binding activity of IRP2 is regulated according to iron status, whereas IRE-binding activity of IRP1 does not change. Most IRP1 is in a non–IRE-binding form that can be recruited in vitro by +2ME. (**D**) Cytosolic aconitase activities are equal in iron-deficient and iron-replete animals.

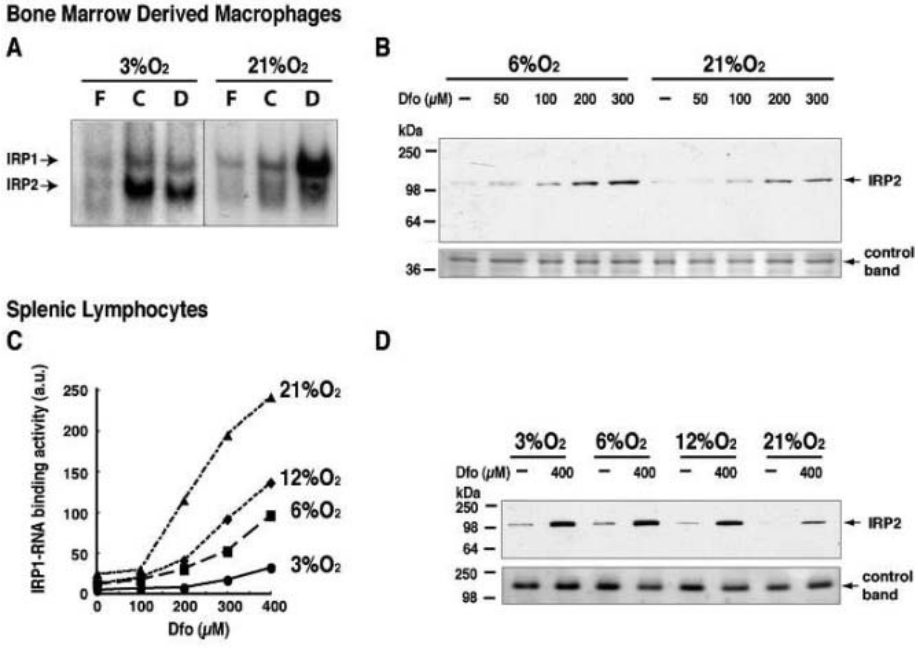

Fig. 2. IRP2 is an effective iron sensor in macrophages and lymphocytes grown at oxygen concentrations ranging from 3 to 21%, whereas IRP1 is a poor sensor at low oxygen concentrations. (**A**) The ratio of IRE-binding activities of IRP1 and IRP2 in macrophages, determined by gel retardation assay, shifts markedly when oxygen concentrations change. After 6 days of growth at 3% O_2, macrophages were exposed for 48 hours to either 3 or 21% O_2 and to iron treatments as described in Fig. 1A. (**B**) IRP2 levels are highly regulated in immunoblot assays of primary WT macrophages at both 6 and 21% O_2 concentrations. After cells were incubated for 6 days at 6% O_2, they were exposed for 48 hours to either 6 or 21% O_2 and treated with a constant amount of 100 μM FAC and increasing amounts of Dfo. To verify even protein loading, we stained immunoblot membranes with PonceauS, and a typical band from this gel is shown. (**C**) IRP1 is a good iron sensor at 21% O_2, but its range of regulation at 3 and 6% O_2 is much lower in lymphocytes. Primary splenic lymphocytes were isolated from WT animals and immediately incubated at 3, 6, 12, and 21% O_2 concentrations for 24 hours in the constant presence of 200 μM FAC and increasing amounts of Dfo. Gel-shift assays were performed to assess IRE-binding activities (fig. S1). IRP1-binding activity was quantified on a Typhoon 9200 Imager (Molecular Dynamics) with Image Quant software. (**D**) IRP2 levels are highly regulated in lymphocytes at multiple oxygen concentrations. Western blots of IRP2 from splenic lymphocytes that were incubated for 72 hours in the constant presence of 200 μM FAC and no Dfo or 400 μM Dfo were probed with antibody to IRP2 as described (*9*). Loading control is the same as in (**B**).

increase TfR expression when grown at 3% O_2. However, at 21% O_2, iron-deficient IRP2$^{-/-}$ macrophages almost fully regulated ferritin and TfR expression, suggesting that the regulatory activity of IRP1 at 21% O_2 allowed IRP1 to substitute for IRP2 in regulation of IRP targets.

Increased ferritin and decreased TfR levels were observed in IRP2$^{-/-}$ macrophage immunoblots grown in 3% O_2 (Fig. 3, A and B) and were attributable to changes in the biosynthetic rates of ferritin and TfR grown at low oxygen concentrations (Fig. 3C). The observation that ferritin and TfR regulation were normal in IRP1$^{-/-}$ macrophages maintained at 3% O_2 implied that IRP2 provided almost all of the regulatory activity in macrophages with minimal contribution from IRP1 at these oxygen concentrations. Levels of IRP2 did not change in IRP1$^{-/-}$ macrophages compared to WT at 3% O_2 (Fig. 3D).

Unlike macrophages, which relied almost exclusively on IRP2 for regulation at physiologically relevant oxygen concentrations, lymphocytes appeared to depend on both IRP1 and IRP2 for normal regulation. The ability to regulate ferritin was significantly reduced in IRP2$^{-/-}$ lymphocytes (Fig. 4A). In IRP1$^{-/-}$ lymphocytes grown at 21% O_2, ferritin levels were also significantly elevated compared to levels in WT controls, and in IRP1$^{-/-}$ lymphocytes grown at 6% O_2, ferritin was slightly elevated at the lowest iron concentration points. These results revealed that IRP1 contributes a substantial fraction of IRE-binding activity in lymphocytes, even though it is minimally sensitive to changes in iron status at low oxygen concentrations (Fig. 2C), similar to its previously observed role in brown fat and kidney (*9*). In lymphocytes, IRP2 levels increased in IRP1$^{-/-}$ cells (Fig. 4B), perhaps because loss of IRP1 resulted in a slight increase in ferritin expression (Fig. 4A) that produced a state of subtle functional cytosolic iron deficiency and a concomitant decrease in the rate of iron-dependent IRP2 degradation (*5*, *6*). The increase in IRP2 allowed cells and tissues to partially compensate for loss of IRP1, as was observed in lymphocytes (Fig. 4B) and in tissues of IRP1$^{-/-}$ mice (*9*). Thus, IRP1 contributes significantly to baseline regulation of iron metabolism in some cells, consistent with the increased severity of neurodegeneration in IRP1$^{+/-}$ IRP2$^{-/-}$ animals (*16*), and the embryonic lethality of IRP1$^{-/-}$ IRP2$^{-/-}$ mice (*17*).

IRP1 and IRP2 operate on a continuum in which the partial pressure of oxygen determines relative activity. IRP1 contains an oxidation-sensitive Fe-S cluster that is enzymatically assembled in cells that have sufficient iron and sulfur (*18*, *19*). However, once the Fe-S cluster of IRP1 is assembled, it may remain stable unless it is exposed to oxidants, including oxygen, superoxide, hydrogen peroxide, nitric oxide, and peroxynitrite (*4*, *20*–*23*). At the ambient oxygen concentrations in tissues of healthy animals (*13*–*15*), the Fe-S cluster of IRP1 appears to be stable, and IRP1 is therefore poorly suited to function as an iron sensor (*9*). In contrast, IRP2 has a different mechanism of iron sensing that depends on iron-dependent degradation (*5*, *6*, *24*–*27*). Iron-dependent turnover is intact at physiologically relevant oxygen concentrations, which enables IRP2 to dominate normal regulation of iron homeostasis in mammals.

IRP1 plays a minor role in the regulation of iron metabolism by contributing a fraction of

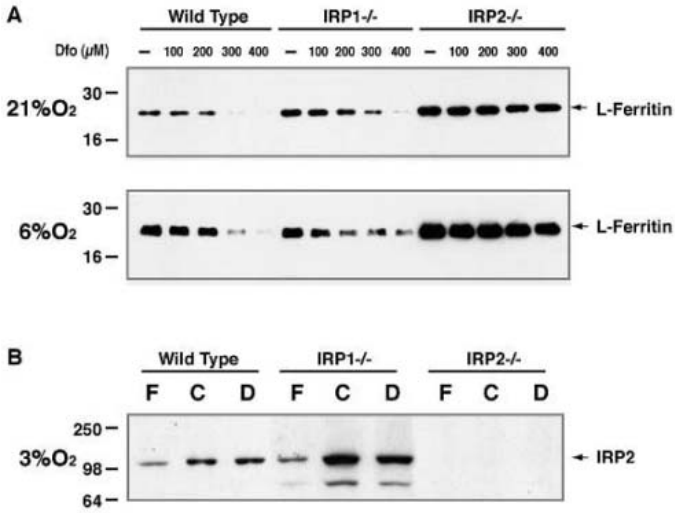

Fig. 4. Misregulation of ferritin occurs in IRP2$^{-/-}$ lymphocytes and to a lesser extent in IRP1$^{-/-}$ lymphocytes, where IRP2 levels increase in compensation for loss of IRP1. (**A**) Lymphocytes were harvested from animals of the different genotypes and grown for 24 hours in 21 or 6% O_2 in the constant presence of 200 μM FAC and various amounts of Dfo. (**B**) In immunoblots on lysates from WT, IRP1$^{-/-}$, and IRP2$^{-/-}$ lymphocytes, IRP2 levels increase substantially in IRP1$^{-/-}$ lymphocytes compared to WT levels. Lymphocytes were grown at 3% O_2 and treated with FAC and Dfo as described in Fig. 3A. Controls for (A) and (B) are given in fig. S3.

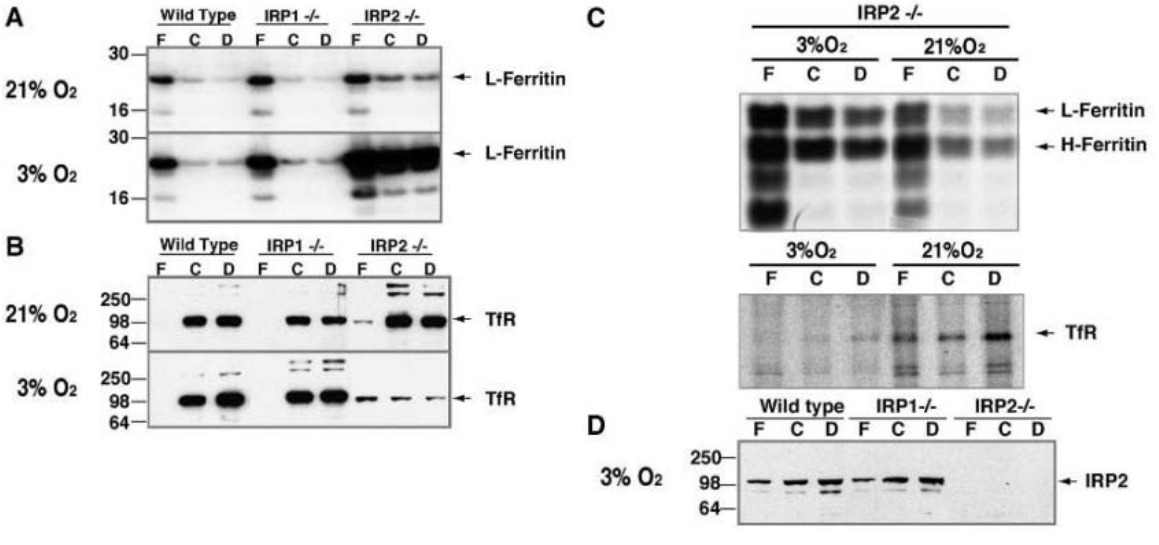

Fig. 3. Misregulation of ferritin and TfR occurs in IRP2$^{-/-}$ macrophages grown at 3% O_2 but not in IRP1$^{-/-}$ macrophages. (**A**) Macrophages were grown for 8 days at either 21 or 3% O_2 and, for the last 48 hours, in either unsupplemented medium (lane C), medium supplemented with 300 μM FAC (lane F), or 100 μM Dfo (lane D). Ferritin levels were detected by immunoblot as described (*30*). (**B**) Levels of TfR were detected by immunoblot in macrophages treated as in (A). (**C**) Ferritin and TfR biosynthesis were assessed by metabolic labeling and immunoprecipitation of ferritin and TfR in macrophages grown at 3% O_2 and treated with FAC and Dfo as described in (A). (**D**) IRP2 levels were assessed by immunoblot in macrophages grown at 3% O_2 and treated with FAC and Dfo as described in (A). Controls for (A), (B), and (D) are given in fig. S2.

baseline IRE-binding activity in tissues. However, the observation that IRE-binding activity can be recruited by oxidative disassembly of its Fe-S cluster means that in some pathological situations, including those in which inflammation results in release of reactive oxygen species, the vast reservoir of IRP1 in the aconitase form (Fig. 1C) may be converted to the IRE-binding form (*20*, *21*). This can lead to inappropriate repression of ferritin synthesis, increased TfR expression, and cellular iron toxicity. Increased iron content coupled with decreased ferritin has been observed in degenerating regions of the brain in Parkinson's disease (*28*). Thus, pathologic activation of IRP1 by reactive oxygen species may play an important role in some disease processes.

Our findings provide genetic evidence that in mammals, the tissue oxygen concentrations are a critical variable in regulating genes that are important in iron metabolism. Results obtained from tissue culture cells grown in room air may lead to conclusions that are not relevant to normal physiology. Iron- and oxygen-based chemistries affect many cellular processes, including mitochondrial function, DNA replication, and the response to hypoxia (*29*). Our work demonstrates that the IRP-regulatory system has evolved to tightly regulate iron metabolism at oxygen concentrations that exist in normal mammalian tissues. Even though the two IRPs are highly homologous (*1*), their activities are only partially redundant, and they occupy different regulatory niches. In normal physiology, tissue oxygen tension determines the contribution of each IRP to the regulation of iron homeostasis.

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30. Materials and methods are available as supporting material on *Science* Online.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/306/5704/2087/DC1
Materials and Methods
Figs. S1 to S3
References

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Hepcidin Regulates Cellular Iron Efflux by Binding to Ferroportin and Inducing Its Internalization

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Hepcidin is a peptide hormone secreted by the liver in response to iron loading and inflammation. Decreased hepcidin leads to tissue iron overload, whereas hepcidin overproduction leads to hypoferremia and the anemia of inflammation. Ferroportin is an iron exporter present on the surface of absorptive enterocytes, macrophages, hepatocytes, and placental cells. Here we report that hepcidin bound to ferroportin in tissue culture cells. After binding, ferroportin was internalized and degraded, leading to decreased export of cellular iron. The posttranslational regulation of ferroportin by hepcidin may thus complete a homeostatic loop: Iron regulates the secretion of hepcidin, which in turn controls the concentration of ferroportin on the cell surface.

The liver-produced hormone hepcidin controls plasma iron levels by regulating the absorption of dietary iron from the intestine, the release of recycled hemoglobin iron by macrophages, and the movement of stored iron from hepatocytes [for a review, see (*1*, *2*)]. During pregnancy, fetal hepcidin controls the transfer of maternal iron across the placenta to the fetus. In turn, hepcidin levels are homeostatically regulated by hepatic iron and by the need for erythropoiesis as sensed by liver oxygenation. Hepcidin is also induced during inflammation, in which hepcidin's effect on iron transport causes the characteristic decrease in blood iron (hypoferremia of inflammation). The hypoferremia is thought to increase host resistance to microbial infection but also leads to the anemia of inflammation (often referred to as the anemia of chronic disease).

Ferroportin (Fpn) is an iron exporter on the surface of absorptive intestinal enterocytes, macrophages, hepatocytes, and placental cells, all of which release iron into plasma (*3*–*5*). To determine whether hepcidin interacts with Fpn, we generated a stable cell line (HEK293-Fpn) expressing mouse Fpn with a C-terminal green fluorescent protein (GFP) under the control of the ecdysone-inducible promoter. In the absence of the inducer ponasterone, there was no detectable synthesis of Fpn-GFP. Within 24 hours of ponasterone addition, there was abundant GFP fluorescence outlining the surface of cells (fig. S1). To determine whether Fpn-GFP was functional, we examined the effect of ferroportin induction on cellular iron levels, as measured by the accumulation of ferritin, the cytosolic iron storage protein. Incubation of cells with ferric ammonium citrate (FAC) alone resulted in a large increase in ferritin levels. FAC loading with simultaneous induction of Fpn-GFP prevented ferritin accumulation (Fig. 1A). Similar results were obtained when diferric transferrin was used as an iron source and levels of IRP2, inversely regulated by cytosolic iron (*6*, *7*), were measured as an indicator of cellular iron levels (fig. S2). Induction of Fpn-GFP resulted in an increase of IRP2 levels when compared to uninduced cells, indicating that cytosolic iron levels decreased after the induction of Fpn-GFP. To show that reduced cytosolic iron levels re-

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sulted from Fpn-induced iron export rather than inhibition of iron uptake, cells were first incubated with FAC and then induced to express Fpn-GFP in the continued presence of FAC. Even with high levels of FAC in the medium, induction of Fpn resulted in a decrease in ferritin levels (Fig. 1B).

The addition of hepcidin to Fpn-GFP–expressing cells markedly changed the distribution of Fpn-GFP from the cell surface to punctate intracellular vesicles (Fig. 2A). Internalization of Fpn-GFP by hepcidin was observed even in the face of continued synthesis of Fpn-GFP. In the presence of the protein synthesis inhibitor cycloheximide, Fpn-GFP remained on the cell surface (fig. S3A), and only the addition of hepcidin caused the loss of surface Fpn-GFP and the appearance of Fpn-GFP in intracellular vesicles (fig. S3B). When hepcidin was removed from the medium, there was no recovery of cell surface fluorescence in the absence of proteins synthesis. Thus, once Fpn-GFP is internalized by hepcidin, it does not recycle to the cell surface.

Concentrations of hepcidin as low as 0.1 μM (0.3 μg/ml) induced Fpn internalization within 1 hour, whereas concentrations 10 times lower resulted in Fpn internalization over a 3-hour time course. These values are consistent with estimates of plasma hepcidin concentration based on urinary hepcidin excretion in either iron-loaded or infected individuals (*8*, *9*). Chemically synthesized hepcidin was as efficient in inducing Fpn-GFP internalization as was hepcidin purified from urine. Because hepcidin is a small cationic peptide, we considered the possibility that hepcidin-induced internalization of Fpn-GFP was nonspecific, because cationic peptides and proteins are known to induce endocytosis (*10*). Protegrin is a cationic antimicrobial peptide from pig neutrophils that is structurally similar to hepcidin (18 amino acids, cationic, beta-sheet, but only two disulfides) (*11*). The addition of protegrin to cells had little effect on the distribution of Fpn-GFP (fig. S4). Chemically synthesized truncated hepcidin lacking the five N-terminal residues (hep20) but retaining hepcidin conformation and most of the cationic residues (*12*) also had no effect on Fpn distribution or protein levels. Thus, hepcidin-induced Fpn-GFP internalization was specific for bioactive hepcidin (hep25).

Hepcidin had no effect on the distribution of another membrane receptor, epidermal growth factor receptor (EGFR) (Fig. 2B). Because EGFR can be better visualized in HeLa cells than in HEK293-Fpn cells, HeLa cells were transiently transfected with a plasmid expressing Fpn-GFP under the control of the cytomegalovirus (CMV) promoter. The addition of EGF, which induced the internalization of EGFR, had no effect on the distribution of Fpn-GFP, whereas the addition of hepcidin resulted in the internalization of Fpn-GFP but not EGFR.

Hepcidin induced not only internalization but also degradation of Fpn-GFP. In HeLa cells transfected with a plasmid containing a CMV-regulated Fpn-GFP, internalized Fpn-GFP colocalized with Lamp-1, a late endosomal/lysosomal marker (fig. S5). The addition of hepcidin to HEK293-Fpn cells for 4 hours caused the loss of Fpn-GFP (Fig. 3, A and B, and fig. S6). Chloroquine, an alkalinizing agent that inhibits lysosomal protease activity, prevented the hepcidin-induced loss of Fpn-GFP and increased Fpn-GFP fluorescence in intracellular vesicles, many of which colocalized with the lysosomal marker Lamp-1. Hep20 did not induce degradation of Fpn-GFP (fig. S6). These results demonstrate that Fpn-GFP internalized by hep25 is degraded in lysosomes.

We next examined whether hepcidin-mediated Fpn-GFP internalization affected iron transport. In the absence of hepcidin, induction of Fpn-GFP in cells exposed to FAC prevented the accumulation of ferritin, but the addition of increasing concentrations of hepcidin progressively increased cellular ferritin levels (Fig. 3C). Protegrin did not affect ferritin levels. Similarly, when cells were loaded with Tf(^{59}Fe)$_2$ and cellular iron levels were measured by the accumulation of radioactivity, induction of Fpn-GFP led to decreased radioactivity, whereas the addition of hepcidin to cells expressing Fpn-GFP led to an increase in radioactivity (Fig. 3D).

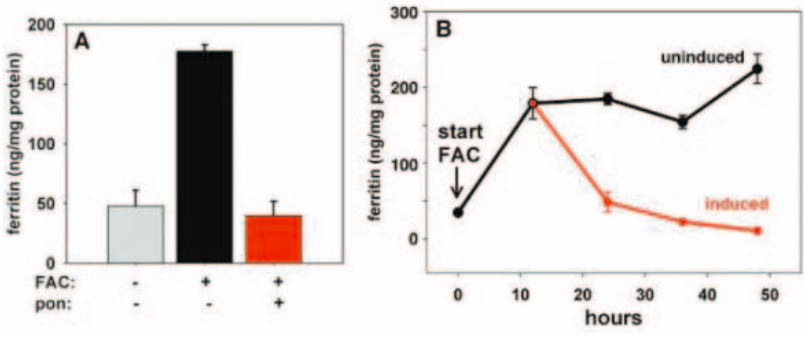

Fig. 1. Expression of a functional cell surface Fpn-GFP. (**A**) EcR293 cells were stably transfected with a plasmid containing an ecdysone-regulated Fpn-GFP construct. The resulting HEK293-Fpn cells were incubated with 10 μM FAC in the absence or presence of 10 μM ponasterone (pon) for 24 hours. Cells were harvested, and ferritin content was determined by enzyme-linked immunosorbent assay (ELISA). FAC addition resulted in increased ferritin, which was prevented by the simultaneous addition of ponasterone. (**B**) Cells were incubated with FAC for 12 hours. Ponasterone was then added to one set of cells (red line), and the cells were incubated in the continued presence of FAC for an additional 48 hours. Induction of Fpn-GFP decreased ferritin even in the presence of iron.

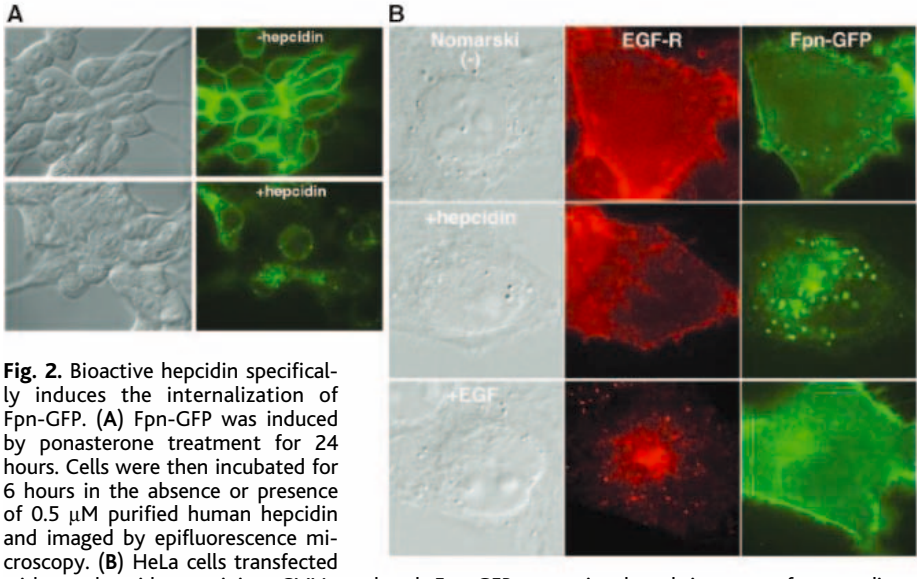

Fig. 2. Bioactive hepcidin specifically induces the internalization of Fpn-GFP. (**A**) Fpn-GFP was induced by ponasterone treatment for 24 hours. Cells were then incubated for 6 hours in the absence or presence of 0.5 μM purified human hepcidin and imaged by epifluorescence microscopy. (**B**) HeLa cells transfected with a plasmid containing CMV-regulated Fpn-GFP were incubated in serum-free medium overnight and then in the presence of cycloheximide (75 μg/ml) for 2 hours. In the continued presence of cycloheximide, cells were incubated, either with media alone (–), hepcidin (0.5 μM), or EGF (0.16 μM) for an additional 4.5 hours. The cells were processed for immunofluorescence and stained with a rabbit antibody to EGFR, followed by an Alexa 594 (red)–conjugated goat antibody to rabbit. Nomarski indicates the type of optics used to image the cells with a halogen lamp.

Hepcidin had no effect on uninduced cells. Thus, hepcidin prevents iron export by removing Fpn from the cell surface, which leads to cellular retention of iron.

We then determined whether hepcidin caused Fpn-GFP internalization by directly binding to Fpn. Human hepcidin contains two histidines that can be iodinated. The efficiency of hepcidin radioiodination, however, was low (specific activity 10^5 to 10^6 cpm/μg). We generated a modified hepcidin with a $Met^{21}\rightarrow Tyr^{21}$ substitution because tyrosine is present in this position in zebrafish hepcidin (GenBank accession number AAP80240). The modified hepcidin was as effective in inducing Fpn-GFP internalization as the unmodified peptide and allowed for a greatly increased efficiency of radioiodination (specific activity 5×10^7 cpm/μg). Cells expressing Fpn-GFP showed increased binding of ^{125}I-hepcidin relative to uninduced cells (Fig. 4A). Nonradioactive hepcidin competed with ^{125}I-hepcidin for binding to Fpn-GFP–expressing cells (with a median inhibitory concentration of about 700 nM), but protegrin or truncated hepcidin lacking the first five amino terminal residues (hep20) did not.

Cross-linking studies also indicated that hepcidin binds directly to Fpn. ^{125}I-hepcidin was cross-linked to cells uninduced or induced to express Fpn-GFP, and cellular lysates were either analyzed directly (Fig. 4B) or immunoprecipitated with an antibody to GFP (Fig. 4C). In both cases, ^{125}I-hepcidin was found in a band of the expected size for a complex of hepcidin–GFP-Fpn in ponasterone-induced but not -uninduced cells. No signal was detected when excess unlabeled hepcidin was added with iodinated hepcidin. The addition of protegrin did not prevent the binding of iodinated hepcidin. Thus, Fpn acts as the hepcidin receptor.

An inverse relation has been observed between hepcidin levels and Fpn protein or mRNA (*13*–*16*), but the mechanism underlying this relation is unclear. Most studies have focused on factors that affect Fpn mRNA levels. Fpn has a putative 5′ iron-responsive element (IRE), suggesting the possibility of regulation by the iron-binding proteins IRP1 and IRP2. Fpn protein levels, however, are inversely correlated with hepcidin mRNA even in mice with a mutation in the 5′ IRE of Fpn (*15*). Here we have found that Fpn levels can be regulated by direct interaction with hepcidin, resulting in the internalization and degradation of Fpn. The use of inducible or constitutive promoters that are iron-independent clearly separates the loss of Fpn from any effect on Fpn synthesis. Forced expression of Fpn in cultured cells results in a profound decrease in cytosolic iron (*3*), suggesting that the iron transport activity of Fpn is constitutive. Cellular iron export may be controlled by the concentration of Fpn at the cell surface, either through synthesis or, as shown here, through ligand-induced internalization and degradation.

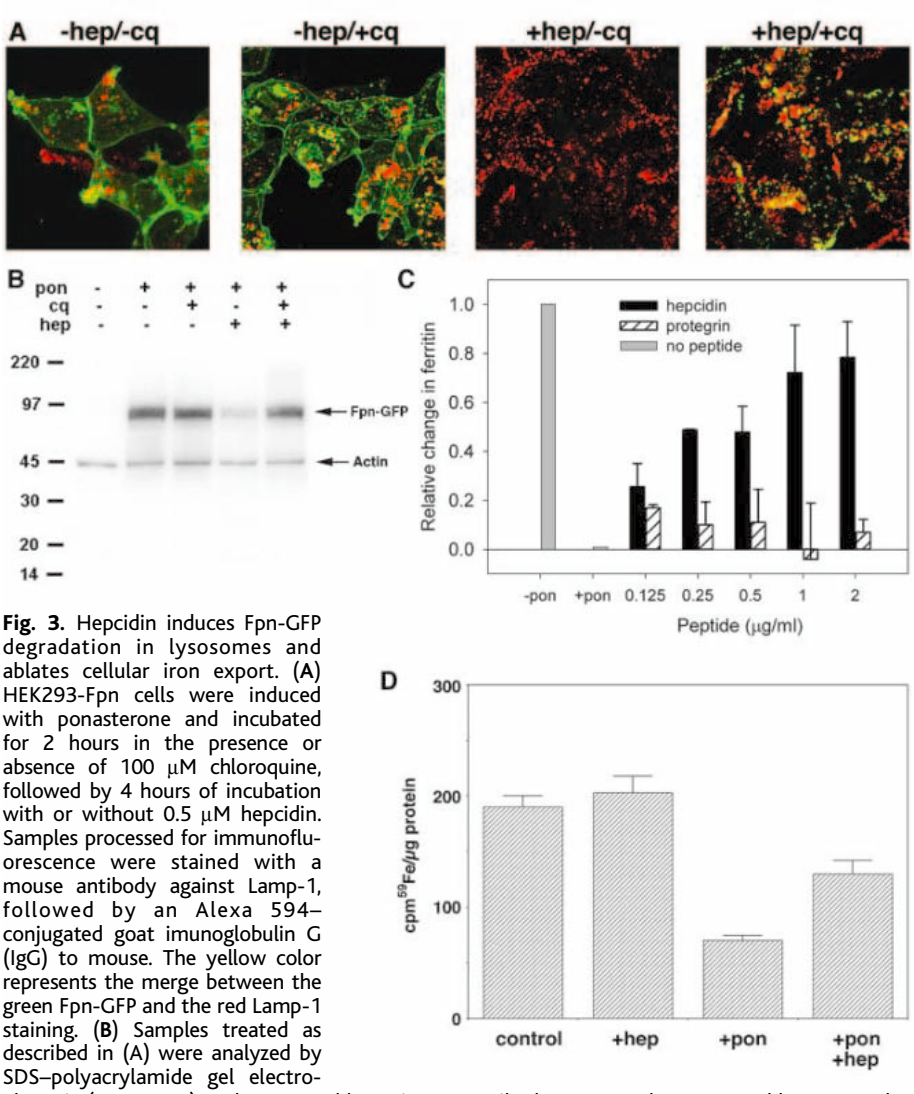

Fig. 3. Hepcidin induces Fpn-GFP degradation in lysosomes and ablates cellular iron export. (**A**) HEK293-Fpn cells were induced with ponasterone and incubated for 2 hours in the presence or absence of 100 μM chloroquine, followed by 4 hours of incubation with or without 0.5 μM hepcidin. Samples processed for immunofluorescence were stained with a mouse antibody against Lamp-1, followed by an Alexa 594–conjugated goat imunoglobulin G (IgG) to mouse. The yellow color represents the merge between the green Fpn-GFP and the red Lamp-1 staining. (**B**) Samples treated as described in (A) were analyzed by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot using an antibody to GFP. The Western blots were also probed with an antibody to actin to control for protein loading. (**C**) HEK293-Fpn cells were incubated for 24 hours with FAC. The cells were then induced with ponasterone in the presence or absence of the specified concentrations of hepcidin or protegrin for 24 hours. Cellular protein was extracted, and ferritin were concentrations determined by ELISA. The differences between hepcidin's and protegrin's effect on ferritin accumulation were statistically significant at each dose ($P < 0.01$ as determined by *t* test, except for the lowest dose $P < 0.05$). (**D**) Cells were incubated with 2.5×10^{-8} M Tf(^{59}Fe)$_2$ in the absence or presence of ponasterone or hepcidin (0.7 μM) for 12 hours. Cells were washed and cell-associated radioactivity was determined and normalized for total protein concentration.

Coupling the internalization of Fpn to hepcidin levels could generate a homeostatic loop regulating iron plasma levels and the tissue distribution of iron. Increased plasma iron, from macrophage recycling of aged red blood cells or from intestinal absorption of iron, stimulates hepatocytes by an as yet unknown mechanism to produce more hepcidin. Circulating hepcidin can bind to Fpn, cause its internalization, and trap iron in hepatocytes, macrophages, and absorptive enterocytes. The consequent rise in cytoplasmic iron could reduce iron uptake in these cells (*17*). Continued utilization of plasma iron, predominantly for hemoglobin synthesis by red cell precursors in the bone marrow, would rapidly deplete plasma iron, restoring the system to a steady state.

The hepcidin-ferroportin interaction may be central to the pathophysiology of hereditary hemochromatosis and the anemia of inflammation. Most types of hemochromatosis are characterized by hepcidin deficiency (*18*) or, less frequently, by autosomal dominant mutations of ferroportin (*18*). At the opposite end of the spectrum, in the anemia of inflammation, cytokine-stimulated hepcidin excess restricts the supply of iron for red cell production (*9*, *19*). Detailed analysis of hepcidin-Fpn interactions and Fpn internal-

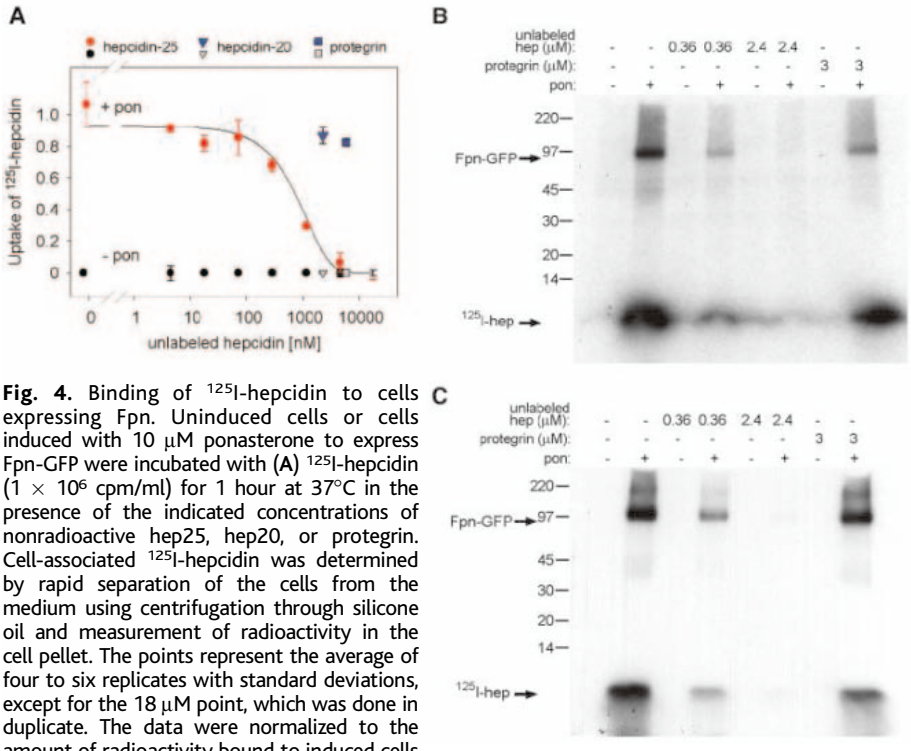

Fig. 4. Binding of ^{125}I-hepcidin to cells expressing Fpn. Uninduced cells or cells induced with 10 μM ponasterone to express Fpn-GFP were incubated with (**A**) ^{125}I-hepcidin (1×10^6 cpm/ml) for 1 hour at 37°C in the presence of the indicated concentrations of nonradioactive hep25, hep20, or protegrin. Cell-associated ^{125}I-hepcidin was determined by rapid separation of the cells from the medium using centrifugation through silicone oil and measurement of radioactivity in the cell pellet. The points represent the average of four to six replicates with standard deviations, except for the 18 μM point, which was done in duplicate. The data were normalized to the amount of radioactivity bound to induced cells in the absence of unlabeled hepcidin (absolute counts were 10,000 to 15,000 cpm), and the amount of radioactivity bound to uninduced cells (1000 to 3000 cpm) was subtracted as background for each point. (**B**) ^{125}I-hepcidin (1.5×10^6 cpm/ml) was added for 15 min at 37°C in the absence or presence of unlabeled hepcidin (hep) or protegrin and was cross-linked by the addition of 5 mM disuccinimidyl suberate (DSS). Cellular lysates (20 μg of total protein) were analyzed by SDS-PAGE and ^{125}I-hepcidin was visualized by autoradiography. Un–cross-linked ^{125}I-hepcidin that dissociated from Fpn during cell lysis is seen at the bottom of the gel. (**C**) Cellular lysates from (B) (400 μg of total protein) were immunoprecipitated with an antibody to GFP, separated by SDS-PAGE and ^{125}I-hepcidin visualized by autoradiography.

ization pathways should provide useful targets for the treatment of these iron disorders.

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Hemoxygenase-2 Is an Oxygen Sensor for a Calcium-Sensitive Potassium Channel

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Modulation of calcium-sensitive potassium (BK) channels by oxygen is important in several mammalian tissues, and in the carotid body it is crucial to respiratory control. However, the identity of the oxygen sensor remains unknown. We demonstrate that hemoxygenase-2 (HO-2) is part of the BK channel complex and enhances channel activity in normoxia. Knockdown of HO-2 expression reduced channel activity, and carbon monoxide, a product of HO-2 activity, rescued this loss of function. Inhibition of BK channels by hypoxia was dependent on HO-2 expression and was augmented by HO-2 stimulation. Furthermore, carotid body cells demonstrated HO-2–dependent hypoxic BK channel inhibition, which indicates that HO-2 is an oxygen sensor that controls channel activity during oxygen deprivation.

Large-conductance, Ca^{2+}-sensitive potassium (BK) channels are strongly implicated in the acute O_2 signaling cascade of a number of cellular systems. In carotid body chemoreceptors (*1*, *2*), low arterial pO_2 is detected by BK channels, and the resulting depolarizing signal is ultimately transduced into increased ventilation. BK channels in pulmonary arteriolar myocytes may contribute to both persistent prenatal (*3*) and acute postnatal hypoxic pulmonary vasoconstriction (*3*, *4*). Hypoxic inhibition of BK channels in perinatal adrenomedullary chromaffin cells is necessary for the catecholamine secretion crucial for preparing the newborn's lung to breathe air (*5*). Hypoxic depression of BK channel activity in neurons of the central nervous system (*6–8*) may also contribute to the excitotoxicity that results from increased neuronal excitability. As O_2 supply becomes compromised, BK channels are acutely and reversibly inhibited (*2*, *7–10*), resulting in cell depolarization. Subsequent voltage-gated Ca^{2+} influx induces hypoxia-dependent neurotransmitter release (*11*). In the carotid body, this ultimately results in increased ventilation. However, the molecular nature

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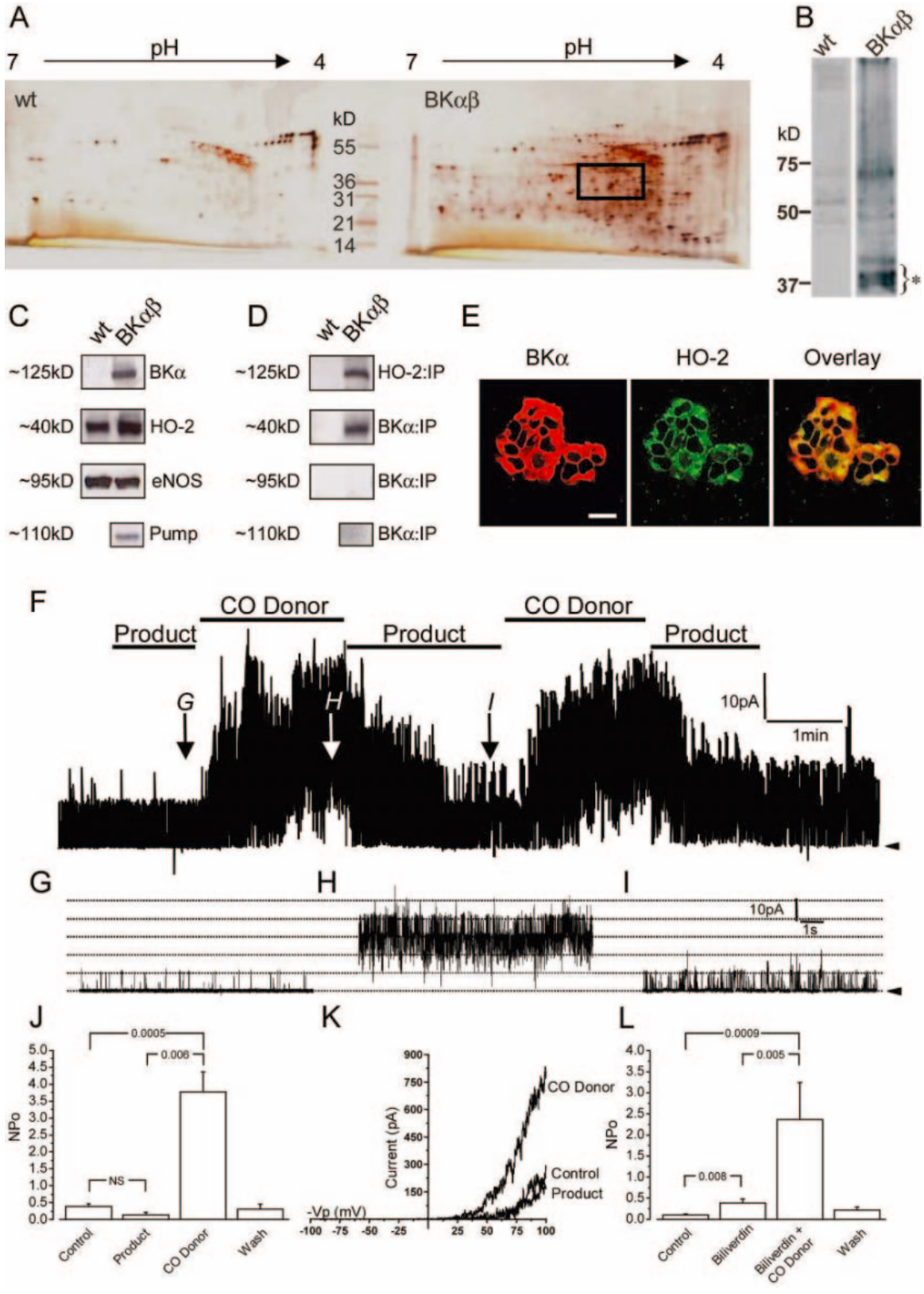

Fig. 1. HO-2 as a functional BKαβ channel–associated protein. (**A**) 2D gel electrophoresis of proteins immunoprecipitated with a BKα antibody from wild-type (wt) and BKαβ-expressing HEK293 cells. Boxed area indicates location of protein spots selected for matrix-assisted laser desorption/ionization–time-of-flight (MALDI-TOF) analysis. (**B**) SDS–polyacrylamide gel electrophoresis (SDS-PAGE) of immunoprecipitates from wt and BKαβ cells. Bands removed for MALDI/TOF analysis are indicated by the asterisk. Linear pH gradients and/or molecular weight markers (in kD) are shown. (**C**) Western blot analyses from lysates of wt and BKαβ cells show that HO-2, eNOS, and α subunit of Na^+,K^+-ATPase (pump) are constitutively expressed. (**D**) Western blot identification of BKα and HO-2 following immunoprecipitation (IP) with the antibodies shown to the right (top two blots). Neither eNOS nor the pump immunoprecipitated with BKα (lower two blots). Pump Western blot and IP were not performed on wt cells. (**E**) Confocal images of BKαβ cells showing colocalization (yellow) of BKα (red) and HO-2 (green) with specific antibodies. Scale bar is 20 μm and applies to all. (**F**) Exemplar current recording from an inside-out membrane patch excised from a BKαβ cell. Periods of application of 30 μM of CO donor and 30 μM of its control (product) shown above trace. Arrows indicate 10-s sections that have been expanded and shown in (**G**), (**H**), and (**I**). (**J**) Mean NPo plot showing effect of 30 μM of CO donor and product on BKαβ channel activity (n = 13 patches). Comparisons between groups are indicated by P values above bars and are from analysis of variance/Bonferroni post hoc test; NS indicates no significant difference. (**K**) Current-voltage relationships showing lack of voltage-dependence of CO activation. (**L**) Mean NPo plot showing effect of 10 μM biliverdin (n = 12 patches) and additive effects of 30 μM CO donor and 10 μM biliverdin (n = 5 patches). Patch potential (–Vp) = +20 mV; $[Ca^{2+}]_i$ = 335 nM.

of the O_2 sensor that regulates BK channels has not been determined. Acute regulation by O_2 of both native (*2*, *8*, *12*) and recombinant (*10*) BK channels is variably retained (*2*, *8*, *6*, *12*), which indicates that the O_2 sensor is either cell-specific or developmentally regulated, or both. Because human recombinant BK channels consisting of both BKα subunits and BKβ subunits (BKαβ) demonstrate O_2 sensitivity in inside-out membrane patches (*10*), the O_2 sensing machinery must be closely associated with the channel protein complex (*8*, *10*, *11*).

To identify proteins associated with recombinant human BK channels, human $BK\alpha_1$ (KCNMA1) and $BK\beta_1$ (KCNMB1) were stably expressed in human embryonic kidney (HEK293) cells (*13*). Proteins were immunoprecipitated with a BKα-specific antibody from lysates of HEK293 cells and separated by two-dimensional (2D) (Fig. 1A, right) and 1D (Fig. 1B, right) gel electrophoresis. Parallel immunoprecipitations were performed on untransfected cells (Fig. 1A, left, and Fig. 1B, left). Of the unique proteins that immunoprecipitated with BKα, peptide mass mapping with mass spectroscopy of trypsin digests consistently identified gamma glutamyl transpeptidase (GGT) and hemoxygenase-2 (HO-2) as associated proteins (table S1). Although GGT associates directly with BKα, it is not involved in hypoxic inhibition of BKαβ channels (*14*). Western blot analyses of cell lysates revealed constitutive expression of HO-2 in the absence or presence of BKαβ (Fig. 1C). The inducible form of hemoxygenase (HO-1) could not be identified in either case (*15*). BKα and HO-2 coimmunoprecipitated only from lysates of BKαβ cells (Fig. 1D). Despite the abundant expression of endothelial nitric oxide synthase (eNOS) and the α subunit of the Na^+- and K^+-dependent adenosine triphosphatase (Na^+,K^+-ATPase) (Fig. 1C), BKα did not immunoprecipitate in a complex with either protein (Fig. 1D). Like HO-2, eNOS is constitutively expressed, is an NADPH (reduced form of nicotinamide adenine dinucleotide phosphate)–dependent enzyme, and produces a short half-life gas. The α subunit of the Na^+,K^+-ATPase is a plasma-membrane protein. Colocalization of BKα with HO-2 was confirmed immunocytochemically by confocal microscopy (Fig. 1E).

In the presence of O_2 and NADPH, hemoxygenases catalyze the breakdown of heme to biliverdin, iron, and CO (*16*). Under normoxia ($pO_2 \approx 150$ mmHg), BKαβ channel activity was reversibly activated by the chemical CO donor, $[Ru(CO)_3Cl_2]_2$. The breakdown product of this compound, $RuCl_2(DMSO)_4$, which does not release CO, did not affect channel activity, which indicates that CO activates BKαβ channels in inside-out membrane patches (Fig. 1, F to L). The CO donor produced a normalized NPo (product of the number of channels and their open-state probability) 15 times that of the control (Fig. 1, F and J), and this effect was apparent at all activating potentials (Fig. 1K and fig. S1). Biliverdin increased BK channel activity by a factor of 4 (Fig. 1L). In inside-out membrane patches treated sequentially with biliverdin and the CO donor, activation was additive, with the CO donor causing a further increase to 28 times as much as the control (Fig. 1L). Wild-type HEK293 cells do not display BK currents (*10*), and no channel activation could be evoked by the CO donor (*17*).

Consistent with earlier reports (*10*, *14*), hypoxia (acute reduction in pO_2 of the intracellular bathing solution to between 15 and 25 mm Hg) resulted in a modest depression in NPo of inside-out patches excised from BKαβ cells (Fig. 2, A to D). Under normoxia, addition of the HO-2 cosubstrates, heme (1 nM) and NADPH (1 μM), evoked an increase in patch NPo

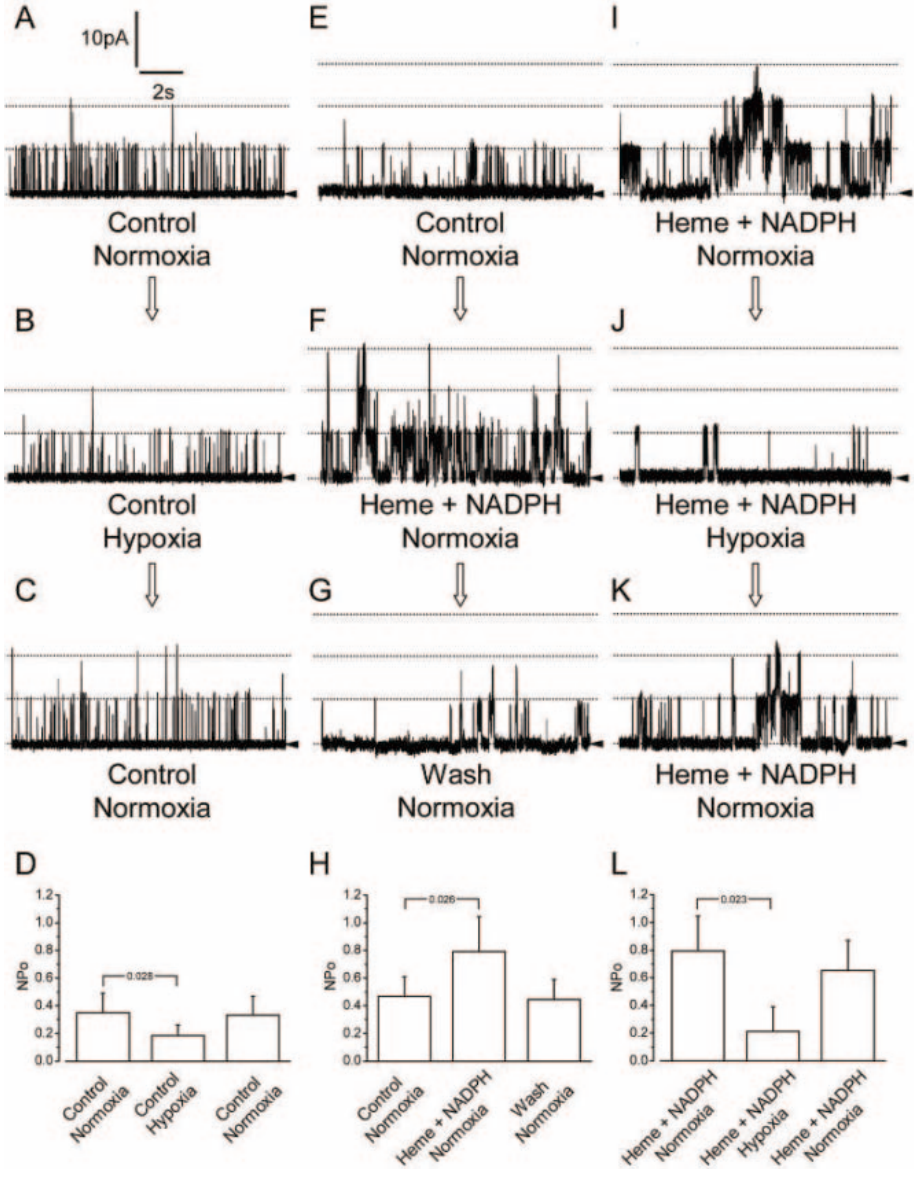

Fig. 2. Hemoxygenase substrates augment BKαβ channel activity and hypoxic inhibition. Exemplar traces and mean NPo plots indicating modest hypoxic channel inhibition in untreated patches (**A** to **D**) (n = 14 patches), increased baseline channel activity by 1 nM heme plus 1 μM NADPH (**E** to **H**) (n = 15 patches), and augmentation of the hypoxic inhibition in the continued presence of heme plus NADPH (**I** to **L**) (n = 10 patches). All traces are from inside-out membrane patches from BKαβ cells. Comparisons between groups are indicated by P values above bars and are from Student's t test. Patch potential (–Vp) = +20 mV; $[Ca^{2+}]_i$ = 335 nM; normoxic $pO_2 \approx 150$ mmHg; hypoxic $pO_2 \approx 15$ to 25 mmHg.

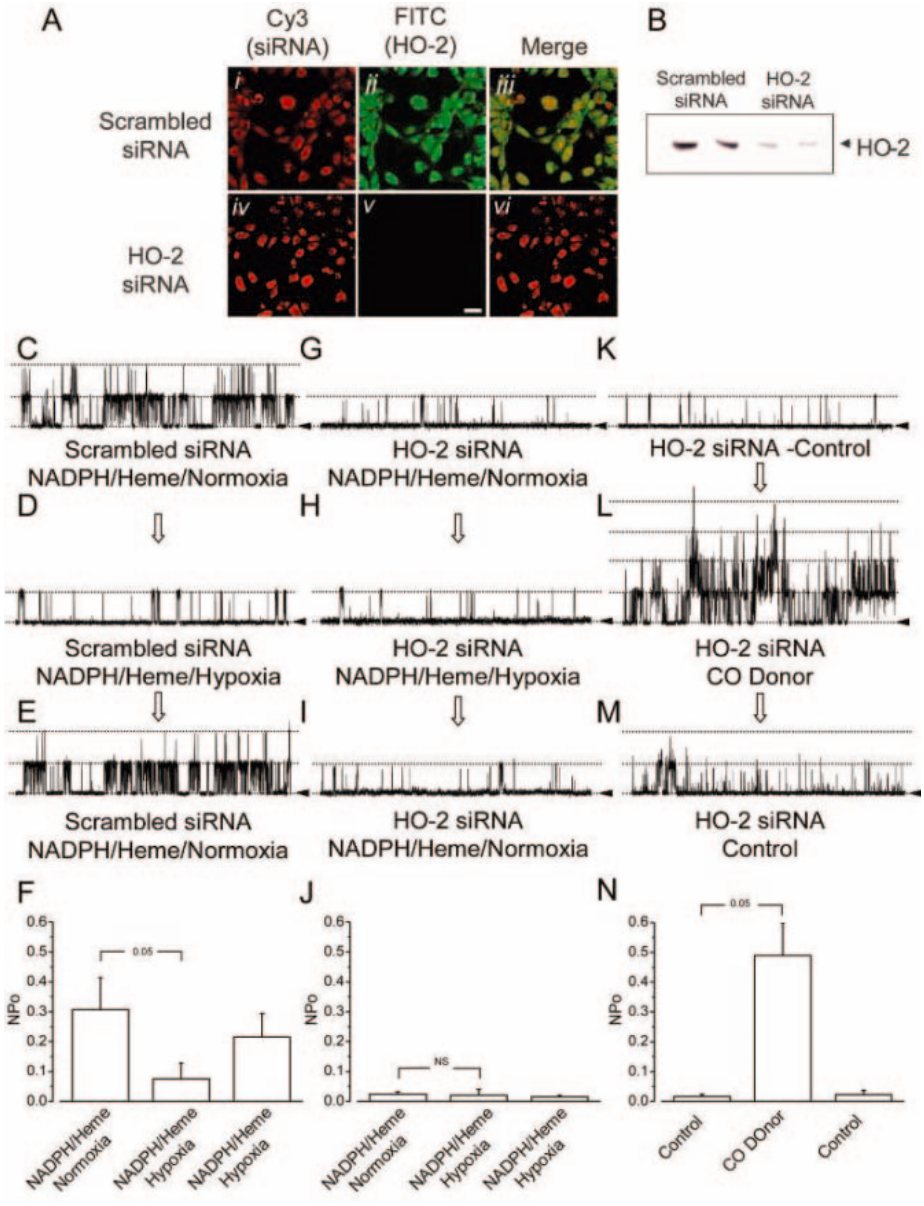

Fig. 3. Modulation of heme- and NADPH-dependent hypoxic inhibition of BKαβ channels after knockdown of HO-2 expression by siRNA. (**A**) Cy3-labeled transfected cells are shown in (A*i*) and (A*iv*). HO-2 immunostaining with a fluorescein isothiocyanate–labeled secondary antibody shows the persistent expression of HO-2 after scrambled siRNA treatment (A*ii*) and knockdown of HO-2 expression after HO-2 siRNA treatment (A*v*). (A*iii*) and (A*vi*) show the merged images. Scale bar in (A*v*) = 20 μm and applies to all. (**B**) Western blot of BKαβ cells transfected with scrambled siRNA (left) and HO-2 siRNA (right) shows ~90% knockdown of HO-2 expression by HO-2 siRNA treatment. Exemplar traces and mean NPo plots of NADPH- and heme-dependent hypoxic BKαβ channel inhibition in scrambled siRNA-treated patches (**C** to **F**) (n = 10 patches), almost complete loss of channel activity in HO-2–treated patches (**G** to **J**) (n = 7 patches), and rescue of channel activity by the CO donor in HO-2–treated patches (**K** to **N**) (n = 3 patches). The siRNA-positive cells were selected by Cy3 fluorescence before patch clamp. Comparisons between groups are indicated by P values above bars and are from Student's t test; NS indicates no significant difference. Patch potential (–Vp) = +20 mV; $[Ca^{2+}]_i$ = 335 nM; normoxic $pO_2 \approx 150$ mmHg; hypoxic $pO_2 \approx 15$ to 25 mmHg.

(Fig. 2, E to H). The 1 nM heme alone does not modulate recombinant BKα channel activity (*18*) or BKαβ channel activity, because NPo in the absence (0.25 ± 0.24) or presence (0.31 ± 0.23) of 1 nM heme were not significantly different from each other (*19*). In the continued presence of the HO-2 cosubstrates, hypoxia evoked a decrease in NPo of more than 70%, which suggests that the enzymatic activity of HO-2 enhances the O_2 sensing ability of the HO-2/BKαβ channel protein complex (Fig. 2, I to L). Thus, O_2 sensing by recombinant human BKαβ channels consists of two components, of which the HO-2–dependent element is the larger of the two.

Selective knockdown of HO-2 expression was achieved by RNA interference. Cells were transfected for 48 hours with Cy3-labeled small interfering (si) RNA designed against either a scrambled human glyceraldehyde phosphate dehydrogenase (GAPDH) coding sequence or the human HO-2 coding sequence. Fluorescence microscopy (Fig. 3A) was used to identify siRNA-positive cells prior to patch clamp. No knockdown of HO-2 immunoreactivity was observed with the scrambled siRNA. In contrast, almost total loss of HO-2 immunoreactivity was achieved with the specific HO-2 siRNA. This was confirmed by Western blot analysis (Fig. 3B). The NADPH- and heme-dependent hypoxic suppression seen in untreated cells (Fig. 2, I to L) was maintained in control siRNA-treated cells (Fig. 3, C to F). Following suppression of HO-2 expression with HO-2 siRNA, mean patch NPo was depressed and NADPH- and heme-dependent hypoxic suppression was absent (Fig. 3, G to J). However, the CO donor rescued this loss of function in all membrane patches tested (Fig. 3, K to N).

BK channel activity present in inside-out membrane patches excised from rat carotid body glomus cells was modestly inhibited by hypoxia (Fig. 4, A to C and G), an effect that is likely to be overestimated because rundown occurred in the absence of HO-2 substrates (Fig. 4G). As is the case with the recombinant system, supplying the channel complex with heme and NADPH (Fig. 4G) or the addition of the CO donor (*20*) under normoxic conditions increased patch NPo. Furthermore, hypoxic inhibition was augmented in the presence of heme and NADPH, which suggests that the HO-2–dependent O_2 sensing system is fully operable in

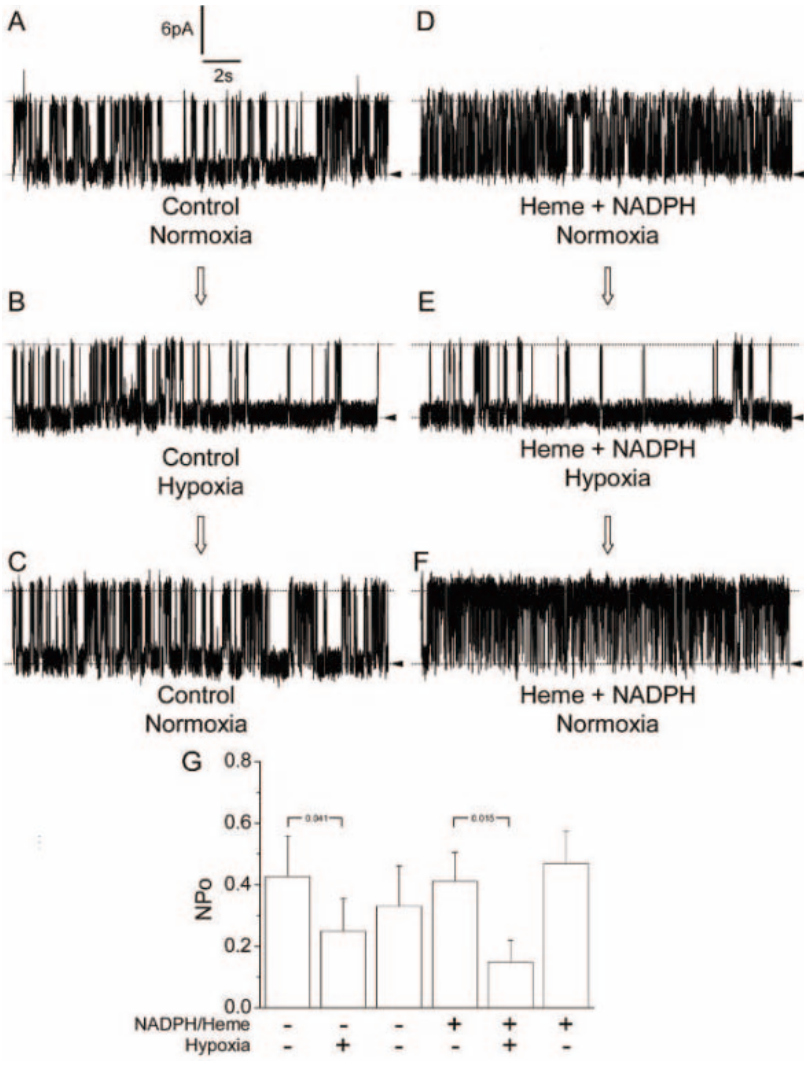

Fig. 4. Augmentation of carotid body glomus cell BK channel activity by hemoxygenase substrates. Exemplar traces indicating the modest hypoxic channel inhibition observed in untreated patches (**A** to **C**) (n = 7 patches), increased baseline channel activity by 1 nM heme plus 1 μM NADPH (**C** and **D**) (n = 7 patches), and augmentation of the hypoxic inhibition in the continued presence of heme/NADPH (**D** to **F**) (n = 7 patches). Corresponding mean NPo values are shown in (**G**). Comparisons between groups are indicated by P values above bars and are from Student's t test. All traces are from inside-out membrane patches excised from carotid body glomus cells. Patch potential (–Vp) = +20 mV; $[Ca^{2+}]_i$ = 335 nM; normoxic $pO_2 \approx 150$ mmHg; hypoxic $pO_2 \approx 15$ to 25 mmHg.

native carotid body glomus cells (Fig. 4, D to G).

HO-2 is highly and constitutively expressed in neuronal and chemosensing tissues, including carotid body glomus cells (*16*, *21*–*23*), whereas HO-1 is not. Functional interaction between HO-2 and BKαβ channels is intact in excised membrane patches, which suggests that their physical interaction is membrane delimited, whether direct or indirect. Either way, a colocalization of BKαβ with HO-2 is necessary for both basal and O_2-dependent activity. Channel activation by CO gas has also been reported in glomus cells, further supporting the notion that HO-2 activity is crucial to native BK channel regulation (*2*). The presence of HO-2 in the BK channel complex provides a molecular explanation for the observation that hemoxygenase inhibition results in carotid body excitation (*23*). Our study supports a model in which O_2 sensing is conferred upon the BK channel by colocalization with HO-2. In normoxia, tonic HO-2 activity generates CO and biliverdin, both of which maintain the open-state probability of the channel at a relatively high level. CO and biliverdin together evoke BK channel activation that is more than additive, representing a means by which the normoxic signal is amplified. However, because biliverdin is rapidly broken down to bilirubin, it seems more likely that the physiological messenger is CO. Whatever the molecular nature of the CO effect, cellular CO levels are reduced during a hypoxic challenge as HO-2 substrate (O_2) becomes scarce, and they rapidly fall below the critical threshold for the maintenance of BK channel activity at the tonically high level. Thus, HO-2 functions as a sensor of acute reduction in environmental O_2 by suppressing both native and recombinant BK channel activity, primarily through the production of CO.

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Supporting Online Material

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Materials and Methods
Fig. S1
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Discovery of a Major D-Loop Replication Origin Reveals Two Modes of Human mtDNA Synthesis

Jennifer Fish,* Nicola Raule,* Giuseppe Attardi†

Mammalian mitochondrial DNA (mtDNA) replication has long been considered to occur by asymmetric synthesis of the two strands, starting at the multiple origins of the strand-displacement loop (D-loop). We report the discovery of a major replication origin at position 57 in the D-loop of several human cell lines (HeLa, A549, and 143B.TK$^-$) and immortalized lymphocytes. The nascent chains starting at this origin, in contrast to those initiated at the previously described origins, do not terminate prematurely at the 3′ end of the D-loop but proceed well beyond this control point, behaving as "true" replicating strands. This origin is mainly responsible for mtDNA maintenance under steady-state conditions, whereas mtDNA synthesis from the formerly identified D-loop origins may be more important for recovery after mtDNA depletion and for accelerating mtDNA replication in response to physiological demands.

Traditionally, mammalian mtDNA replication has been thought to occur through an asymmetric mechanism. This was proposed in a model introduced more than 25 years ago, which held that the two mtDNA strands replicate in partial asynchrony (*1*–*3*). Recently, however, evidence favoring the existence of a bidirectional, strand-coupled mechanism has been presented (*4*–*6*), raising a lively debate (*7*–*9*). The proponents of this alternative model suggest that the multiple origins within the D-loop represent points of fork arrest of bidirectional replication starting downstream. Here, we examine the role of the mtDNA heavy strand (H-strand) replication origins in three human cell lines—HeLa, A549 lung carcinoma, and 143B.TK$^-$ osteosarcoma cells—and in several immortalized lymphocyte cell lines.

To measure the rate of mtDNA replication, we used primer extension to make a complementary copy of each mtDNA nascent H-strand chain present in a preparation of total cell DNA (*10*, *11*). This technique uses VENT DNA polymerase and an appropriate [5′-^{32}P]-labeled light strand (L-strand) oligodeoxynucleotide primer, chosen within the D-loop sequence (internal) or 3′ to this sequence (external) (Fig. 1A). The extended primers are then separated by polyacrylamide gel electrophoresis (PAGE). From the radioactivity associated with extended primers synthesized in vitro on nascent chains, one can estimate the relative amounts of these chains, which should reflect their relative rates of synthesis and, consequently, the relative activities of the corresponding origins.

Figure 2A shows the PAGE patterns of the extended L-strand primers obtained from total cell DNA samples of the three human cell lines with the use of the D-loop–internal primer Ip1 (Fig. 1A). These patterns exhibited species of nascent chains, absent in mtDNA-less 143B.ρ°206 cells (*12*), which corresponded to origins at or very near positions 191, 167, 151, 146, and 110 in the mtDNA sequence (*13*). These origins matched the major initiation sites of H-strand synthesis determined earlier in the mtDNA D-loop of human cells (*14*–*16*). The slowly migrating, highly labeled band observed in Fig. 2 derived mainly from primer extension on the parental H-strand and was thus labeled PSex (parental strand extended) (Fig. 2C) (*11*).

It was previously shown that most of the nascent H-strands originating in the D-loop terminate prematurely at the 3′-end of the loop (*17*), forming the 7S DNA (*1*) (Fig. 1A). To identify the nascent H-strand chains that extended beyond the D-loop, we used the external primer Ep1 (Fig. 1A). The patterns for all three cell lines (Fig. 2B) exhibited a set of extended primers corresponding in size to those expected for nascent chains starting from the various origins previously detected with the internal primer (Fig. 2A); these bands varied in intensity in each cell line and between different cell lines, in a manner similar to what was observed with internal primers. Unexpectedly, the three patterns also showed a strong band representing a new species of extended primers, absent in the pattern from 143B.ρ°206 cell DNA (Fig. 2D), which corresponded to an origin at an mtDNA position near 60 in the D-loop. The same results were obtained with the external primers Ep2 (Fig. 2D), Ep3 (fig. S1), and Ep4 (Fig. 2E).

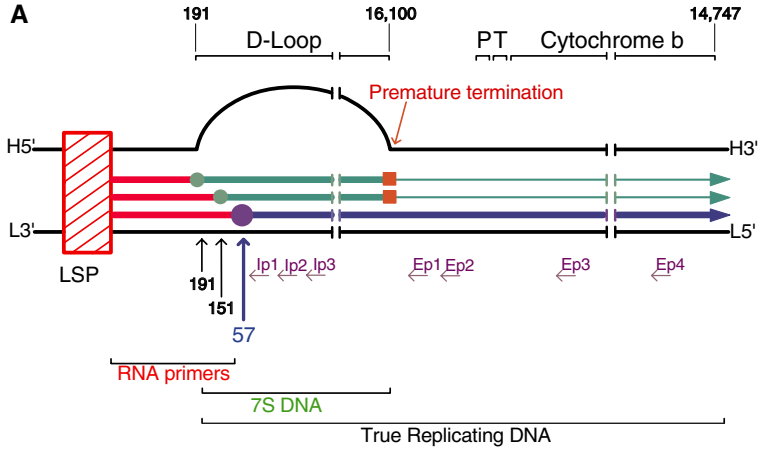

Fig. 1. (**A**) Schematic representation of H-strand replication initiation in human mtDNA D-loop region. P, tRNA^{Pro} gene; T, tRNA^{Thr} gene; H, heavy strand; L, light strand; LSP, L-strand transcription promoter; Ip1 to Ip3, L-strand internal primers 1, 2, and 3; Ep1 to Ep4, L-strand external primers 1, 2, 3, and 4 (*11*). Numbers indicate nucleotide positions according to the human mtDNA sequence (*13*). (**B**) Identification by sequencing of the position-57 origin. The sequence shows a representative 5′-end–3′-end junction in a cloned PCR product of a circularized extended primer. Numbers of clones from mtDNA of different cell lines that exhibited the 5′-end–3′-end junction sequence shown are indicated.

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An extended primer produced by Ip1 and corresponding to an origin at position ~60 could not have been resolved from the primer in the gel shown in Fig. 2A. However, another experiment with a different internal primer (Ip2, Fig. 1A) revealed in HeLa, A549, and 143B.TK$^-$ cells a species of extended primers corresponding in size to the expected one (Fig. 2B). Elution of these extended primers from the denaturing gel, ligation, polymerase chain reaction (PCR) amplification, cloning, and sequencing of the PCR products (fig. S2) showed the position of the new origin to be position 57 (T) in the mtDNA sequence in 13 of 14 clones, 20 of 24 clones, and 9 of 14 clones derived, respectively, from HeLa, A549, and 143B.TK$^-$ cells (Fig. 1B). It has been reported that the mtDNA L-strand segment including T57 occurs in the loop of a very conserved stem-loop structure (*18*). It is also noteworthy that several 3′-ends of the stable mitochondrial RNA-DNA hybrids (replication-priming R-loops) had previously been mapped to positions 61 and 62 (*19*).

To exclude the possibility that the novel origin at position 57 resulted from an artifactual arrest point for VENT DNA polymerase, we subjected a PCR-amplified 808–base pair (bp) fragment [nucleotide (nt) positions 16,026 to 264] of 143B.TK$^-$ mtDNA, thus including the D-loop, to primer extension with the primer Ep1. The expected extended primer of 767 nt was observed (Fig. 2B, lane PCR), but no other band was detected. To obtain further evidence on this point, we conducted an S1 protection experiment that used a [5′-^{32}P]-labeled L-strand mtDNA probe (nt positions 16,528 to 271) (*11*). The pattern obtained showed that, whereas the full L-strand probe exhibited the expected 313-nt size and was completely digested by S1 nuclease (Fig. 3A), after hybridization with partially purified HeLa cell mtDNA and S1 digestion, the S1-resistant probe fragments corresponded to the expected extended L-strand primers (Fig. 3B). The presence of two extra bands among the S1-resistant chains probably reflects partial S1 protection of folded molecules. Most noteworthy, however, was the presence of a band of protected probe corresponding to the extended primer expected for chains originating at position 57 (Fig. 3B). A very slight variability in the position of these protected nascent chains (corresponding to a few nucleotide length differences) was observed from experiment to experiment. This variability most likely reflected some nibbling by the S1 enzyme of the L-strand probe/H-strand nascent chain hybrid (position 57 lies in the middle of a 6-nt AT-rich stretch).

The human cell lines used in this work are well-established cell lines that have been grown for a long time in vitro. However, several freshly isolated immortalized human lymphocyte cell lines also exhibited the new origin (Fig. 3C).

We quantified the radioactivity associated with the extended primers terminating at the position-57 origin, obtained with the

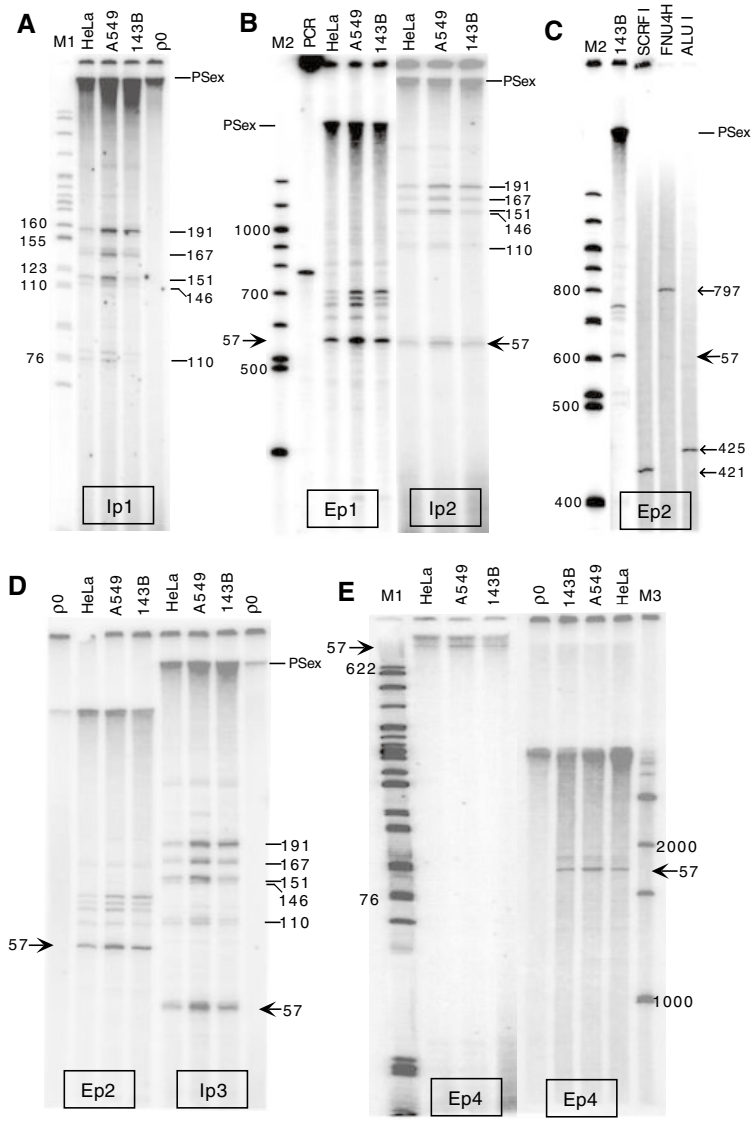

Fig. 2. Identification of a novel D-loop origin of mtDNA nascent H-strand chains. Positions in the human mtDNA sequence (*13*) of the origins are indicated by numbers with tick marks; a large arrow denotes a position-57 origin. (**A**) Primer extension products obtained on all nascent H-strand chains, using as a template the mtDNA present in total cell DNA from the indicated cell lines and, as a primer, the [5′-^{32}P]-labeled L-strand internal primer Ip1. ρ^0, 143B.TK$^-$ $\rho^0$206 cells without mtDNA (*12*); PSex, parental strand extended products. (**B**) Primer extension products obtained on "true" replicating nascent H-strand chains, using the L-strand external primer Ep1 (left gel), or on all nascent H-strand chains, using the L-strand internal primer Ip2 (right gel). The left gel also shows the primer extension products obtained with the Ep1 primer on the PCR-amplified 808-bp mtDNA fragment from 143B.TK$^-$ cells (see text). (**C**) Identification of slow-moving bands as PSex. Total cell DNA from 143B.TK$^-$ cells was subjected to primer extension using the external primer Ep2, and then to PAGE, either directly (lane 143B) or after digestion with the restriction enzymes Scr FI, Fnu 4HI, or Alu I (*24*). The small arrows indicate the labeled fragments expected after cutting the primer extension products with the restriction enzymes. (**D**) Primer extension products obtained on all nascent H-strand chains or on the "true" replicating nascent H-strand chains present in the mtDNA from indicated cell lines, using the internal primer Ip3 or the external primer Ep2, [5′-^{32}P]-labeled to the same specific activity and used in the same ratio to DNA for quantification purposes. (**E**) Primer extension products obtained on "true" replicating nascent H-strand chains from the indicated cell lines, using the external primer Ep4. One portion of the primer extension reaction was run on a 7 M urea–10% polyacrylamide gel for 4 hours at 20 mA (left gel), and another portion on a 7 M urea–5% polyacrylamide gel for 33 hours at 10 mA (right gel). M1, M2, and M3 are DNA markers (*11*).

internal primer Ip3 or the external primer Ep2 (labeled to the same specific activity and used in an identical ratio to the DNA template), in total cell DNA from HeLa, A549, and 143.TK$^-$ cells. The nascent chains corresponding to the position-57 origin had a similar abundance when detected with the internal primer Ip3 or with the external primer Ep2, whereas the species of nascent chains initiating at each of the known origins were detected in much greater amount with the internal than with the external primer (Figs. 1A and 2D). Quantification of the results of several experiments similar to that described above (fig. S3) indicated that the chains initiated at position 57 (hereafter referred to as "O_{57} chains") escape premature termination.

To investigate whether the nascent chains starting at O_{57} extended much beyond the D-loop, we used two new external L-strand primers (Ep3 and Ep4), both within the cytochrome b reading frame. Extended products starting from these primers and corresponding to the earlier identified origins at positions 191, 167, 151, 146, and 110—and, much more abundant, extended primers corresponding to the new origin at position 57—were clearly recognizable in the patterns from HeLa, A549, and 143B.TK$^-$ cells (Fig. 2E) (fig. S1). In HeLa cells, the ratio of "true" replicating O_{57} chains to the cumulative value for all replicating H-strand chains starting at the various D-loop origins (including the position-57 origin), which provides an estimate of the relative contribution to mtDNA maintenance by the position-57 origin, was found to be 71 ± 0.8% for Ep1 or Ep2 (n = 6), ~77% for Ep3 (n = 1) (fig. S1), and 76 ± 0.9% for Ep4 (n = 4) (Fig. 2E). The high proportion of "true" replicating O_{57} chains, as detected with primers complementary to mtDNA sequences located even at large distances from this origin (>1700 bp), justifies the conclusion that these nascent chains are true precursors of the bulk of fully replicated H-strands. The products of Ep4 extension failed to reveal the presence of any possible unidirectional origin in the mtDNA region that encompasses most of the gene encoding cytochrome b (Fig. 2E).

To investigate how the new O_{57} and the previously known origins are regulated after artificially induced mtDNA depletion, we treated HeLa cells and 143B.TK$^-$ cells for 3 days with the specific and reversible mtDNA synthesis inhibitor ethidium bromide (EtBr, 50 ng/ml) (*20*) and then allowed them to recover in drug-free medium for up to 10 days. HeLa cells and 143B.TK$^-$ cells continued to grow at a near-normal rate during the EtBr treatment and during the recovery period (fig. S4, A and B). In both cell lines, quantification of mtDNA by slot blot analysis (*21*) revealed its rapid decrease to <10% of the normal level after 3 days of treatment and its fairly rapid recovery after 5 days of growth in drug-free medium (Fig. 4A) (fig. S5A).

Figure 4B summarizes the results of several experiments carried out on HeLa cells, and fig. S6 shows the patterns obtained in a representative experiment. It appears that the rate of synthesis of the "true" replicating nascent chains initiated at the previously identified origins—which, in naïve cells, represents ~30% of the overall rate of mtDNA replication, and which is totally inhibited in EtBr-treated cells—started increasing almost immediately after drug removal. After 1 day, it reached ~40% of the naïve cell replication rate, accounting for nearly 100% of nascent chains in the recovering cells. After 2 days, it reached ~55% of the naïve cell replication rate, accounting for ~95% of nascent chains in the recovering cells. By contrast, the rate of synthesis of both "true" replicating nascent chains and all nascent chains starting at O_{57} recovered very slowly.

These results indicate a nearly total suppression of premature termination of synthesis at the distal end of the D-loop during

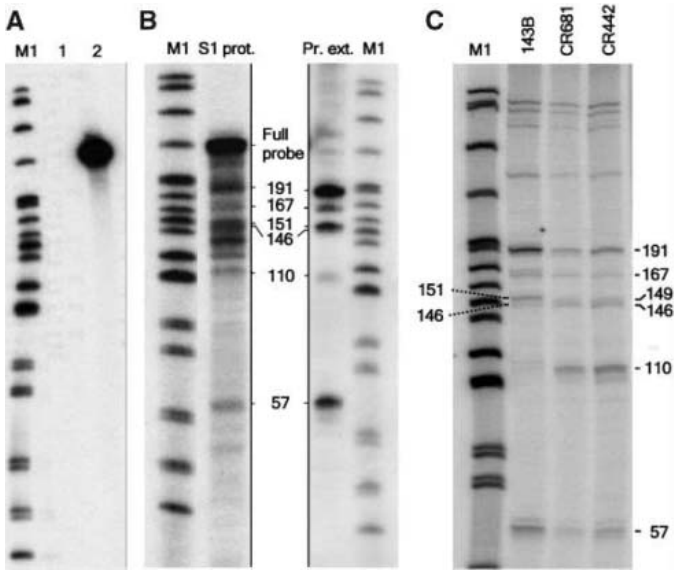

Fig. 3. (**A** and **B**) S1 protection of nascent H-strand mtDNA chains from HeLa cells. (**A**) The purity of the probe was tested before and after digestion with S1 nuclease. Before digestion (lane 2) the probe did not show any impurity; in the absence of added DNA, it was completely digested (lane 1). (**B**) The S1 probe was hybridized with DNA extracted from mitochondria purified by differential centrifugation from HeLa cells, and, after digestion with the S1 enzyme, the protected DNA sample (S1 Prot.) was run on a 7 M urea–10% polyacrylamide gel in parallel with a sample of extended L-strand Ip3 primers (Pr. Ext.) synthesized on nascent H-strand chains from the same cells (*11*). (**C**) Primer extension products obtained on all nascent H-strand mtDNA chains present in 3 μg of total cell DNA from 143B.TK$^-$ cells and two immortalized lymphocyte cell lines from an Italian population (*10*).

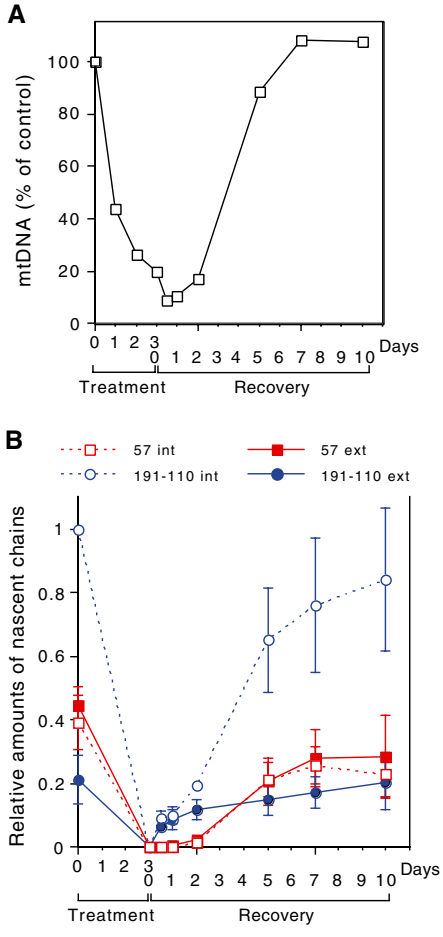

Fig. 4. (**A**) Relative amounts of total mtDNA per cell in HeLa cells during exposure to EtBr (50 ng/ml) for 3 days, or in cells exposed to the drug for 3 days and then transferred to drug-free medium. Average of two determinations is shown. (**B**) Quantification of all nascent H-strand chains (open symbols), as detected with internal Ip3 primer, and of "true" replicating chains (solid symbols), as detected with external Ep2 primer. PAGE conditions were as in Fig. 2. The data for each experiment are expressed relative to the cumulative amount of nascent chains initiated at the 191 to 110 origins, as detected with Ip3 primer before exposure of the cells to EtBr. They represent averages ± SE (n = 3, from two EtBr treatment and recovery experiments). Some error bars were omitted because they were too small.

recovery from mtDNA depletion. The same phenomenon has recently been observed in a mouse cell line after 2′,3′-dideoxycytidine–induced depletion (*22*). Results consistent with those described above were obtained in an experiment that used 2′,3′-dideoxycytidine to induce mtDNA depletion in HeLa cells (fig. S7) and in an experiment with EtBr carried out on 143B.TK$^-$ cells (fig. S5B).

Our data argue against the D-loop origins being sites of fork arrest of a replication initiating downstream of the D-loop. On the contrary, our findings are consistent with an active role of the D-loop–originating chains in mtDNA replication. Therefore, it appears that human cells exhibit two modes of mtDNA replication, each associated with distinct D-loop replication origins. One of these modes—a "maintenance mode" involving the position-57 origin and regulated at the level of this origin—appears to predominate in the maintenance, under steady-state conditions, of the copy number of mtDNA. The other mode—associated with the multiple previously known D-loop origins and regulated at the origins and at the premature termination site at the 3′-end of the D-loop—plays a major role in the initial recovery of the normal mtDNA complement after a depletion. This "induced" mode is possibly also involved in accelerating mtDNA synthesis to satisfy developmental, physiological, or aging-related demands. A decrease in termination has been shown to be responsible for the increased replication rate of mtDNA in proliferating T lymphocytes (*23*).

Although we have not addressed directly the current controversy concerning the mechanism of mammalian mtDNA replication, the evidence presented above has emphasized the importance of the D-loop origins in initiating mtDNA replication, in this respect supporting the original D-loop model.

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Supporting Online Material

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Materials and Methods
SOM Text
Figs. S1 to S7
References

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Phosphorylation of Proteins by Inositol Pyrophosphates

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The inositol pyrophosphates IP_7 and IP_8 contain highly energetic pyrophosphate bonds. Although implicated in various biologic functions, their molecular sites of action have not been clarified. Using radiolabeled IP_7, we detected phosphorylation of multiple eukaryotic proteins. We also observed phosphorylation of endogenous proteins by endogenous IP_7 in yeast. Phosphorylation by IP_7 is nonenzymatic and may represent a novel intracellular signaling mechanism.

Inositol phosphates serve diverse biologic functions, with the best characterized, inositol 1,4,5-trisphosphate (IP_3), mediating the release of intracellular calcium stores. In mammals, inositol pyrophosphates, such as diphosphoinositol pentakisphosphate (5PP-IP_5, or IP_7) and bis-diphosphoinositol tetrakisphosphate ($[PP]_2$-IP_4, or IP_8) (*1–3*), are formed by a family of three evolutionarily conserved inositol hexakisphosphate (IP_6) kinases (IP6Ks) (*4*, *5*). Functions of inositol pyrophosphates include regulation of endocytosis (*6*), chemotaxis (*7*), and apoptosis (*8*).

The standard free energy of hydrolysis of the pyrophosphate bond in IP_7 has been estimated theoretically for the nonphysiological isomer 1PP-IP_5 at 6.6 kcal/mol, higher than that of adenosine 5′-diphosphate (ADP) (6.4 kcal/mol) and lower than that of adenosine 5′-triphosphate (ATP) (7.3 kcal/mol) (*1*). Moreover, the high steric constraints and strong electrostatic repulsion of the vicinal pyrophosphates in the naturally occurring IP_8 isomers (4,5)$[PP]_2$-IP_4 and (5,6)$[PP]_2$-IP_4 suggest that IP_8 would have a standard free energy of hydrolysis much higher than that calculated for IP_7 (*9*). These characteristics suggest that inositol pyrophosphates might serve as phosphorylating agents (*10*).

To determine whether proteins are phosphorylated by IP_7, we used mammalian IP_6 kinase 1 (IP6K1) and $\gamma[^{32}P]$ATP to synthesize IP_7 that was labeled at the β position of the pyrophosphate moiety, $5\beta[^{32}P]IP_7$ (Fig. 1A; fig. S1). To ensure that any apparent phosphorylation does not simply reflect binding of IP_7 to proteins, we synthesized $[^{32}P]IP_7$ labeled at position 2, which is not a pyrophosphate, and $[^{32}P]IP_6$ labeled at position 2 as controls (Fig. 1A). We compared phosphorylation by $5\beta[^{32}P]IP_7$ and $\gamma[^{32}P]$ATP by using equivalent specific activities and molar concentrations of each agent. In both mouse brain and yeast (*Saccharomyces cerevisiae*) extracts, we observed phosphorylation of multiple proteins with $5\beta[^{32}P]IP_7$ but not with either control agent (Fig. 1B). Moreover, incubation of cell extracts with $[^{32}PO_4]_i$ orthophosphate, at the equivalent specific activity and molar concentration as those used for $5\beta[^{32}P]IP_7$, revealed no incorporation of radiolabeled inorganic phosphate into proteins, ruling out the possibility that the phosphorylation by $5\beta[^{32}P]IP_7$ is a reflection of free

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$[^{32}PO_4]_i$ liberated by IP_7 phosphatases present in the cell extracts (Fig. 1B). Under these experimental conditions, $5\beta[^{32}P]IP_7$ phosphorylated as many proteins as $\gamma[^{32}P]ATP$, but most of the labeled proteins were distinct for the two phosphate donors. As endogenous ATP in lysates may dilute $\gamma[^{32}P]ATP$, the observed phosphorylation by ATP may be an underestimate.

Apparent phosphorylation by IP_7 may merely reflect phosphorylation by ATP formed by IP_7 phosphorylation of ADP. However, 1 mM unlabeled ATP or ADP failed to diminish the extent of phosphorylation of mouse brain proteins by $5\beta[^{32}P]IP_7$ (Fig. 1C). Moreover, $5\beta[^{32}P]IP_7$ phosphorylated purified proteins in preparations that lack ADP (see below).

To ascertain the range of proteins phosphorylated by IP_7 in various tissues and species, we examined extracts of *Escherichia coli*, mouse brain, mouse kidney, and *Drosophila melanogaster*. Phosphorylation by IP_7 appears selective for eukaryotic organisms, as no proteins were phosphorylated by $5\beta[^{32}P]IP_7$ in bacterial extracts. In contrast, abundant proteins were phosphorylated in the fly and mouse extracts with a number of differences between kidney and brain extracts (Fig. 1D).

If labeling with $5\beta[^{32}P]IP_7$ reflects protein phosphorylation, then such phosphorylated sites should be subject to dephosphorylation and enhanced by phosphatase inhibitors (Fig. 1E). The phosphatase inhibitor sodium fluoride increased protein phosphorylation by $5\beta[^{32}P]IP_7$ as well as $\gamma[^{32}P]ATP$. Inhibition of IP_7 pyrophosphatases by sodium fluoride (*2*) might also contribute to the observed effect. The more specific phosphatase inhibitors, okadaic acid and tautomycin, also increased protein phosphorylation in the presence of $5\beta[^{32}P]IP_7$, whereas sodium orthovanadate and β-glycerol phosphate had no detectable effects.

Phosphorylation by $5\beta[^{32}P]IP_7$ requires magnesium as a cofactor. Thus, phosphorylation of mouse brain proteins by $5\beta[^{32}P]IP_7$ occurred only in the presence of divalent cations, with magnesium being more effective than manganese (Fig. 1F). Calcium could partially substitute for magnesium. In contrast, phosphorylation by ATP is comparable with manganese and magnesium but almost absent with calcium.

To identify specific proteins phosphorylated by IP_7, we focused on the three most prominent phosphoproteins (at 60, 63, and 98 kD) in yeast extracts. Proteins were partially purified (fig. S2), and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry identified the 60-kD protein as NSR1 [the open reading frame (ORF) YGR159c], a nucleolar protein involved in ribosome assembly and export (*11*, *12*). The 98-kD protein was identified as YGR130c, a protein of unknown function. Both NSR1 and YGR130c that were overexpressed in yeast were phosphorylated by $5\beta[^{32}P]IP_7$ (Fig. 2A). Treatment of extracts with lambda phosphatase, which acts on phosphorylated Ser, Thr, and Tyr residues (Fig. 2B), decreased phosphorylation by $5\beta[^{32}P]IP_7$.

To identify the sites of phosphorylation, we generated deletion mutants of NSR1 for analysis. Phosphorylation occurred predominantly at amino acids 51 to 166 (Fig. 2D), a region containing extensive stretches of Ser residues surrounded by acidic amino acids. A smaller but similar acidic Ser domain is present in YGR130c (fig. S2). The acidic Ser region is critical for phosphorylation, as NAB4 (also know as HRP1, ORF YOL123w), a nuclear polyadenylated RNA-binding protein (*13*) that has RNA-binding sequences similar to those of NSR1 but that lacks the acidic Ser domain, was not phosphorylated by $5\beta[^{32}P]IP_7$ (Fig. 2C). A sequence homol-

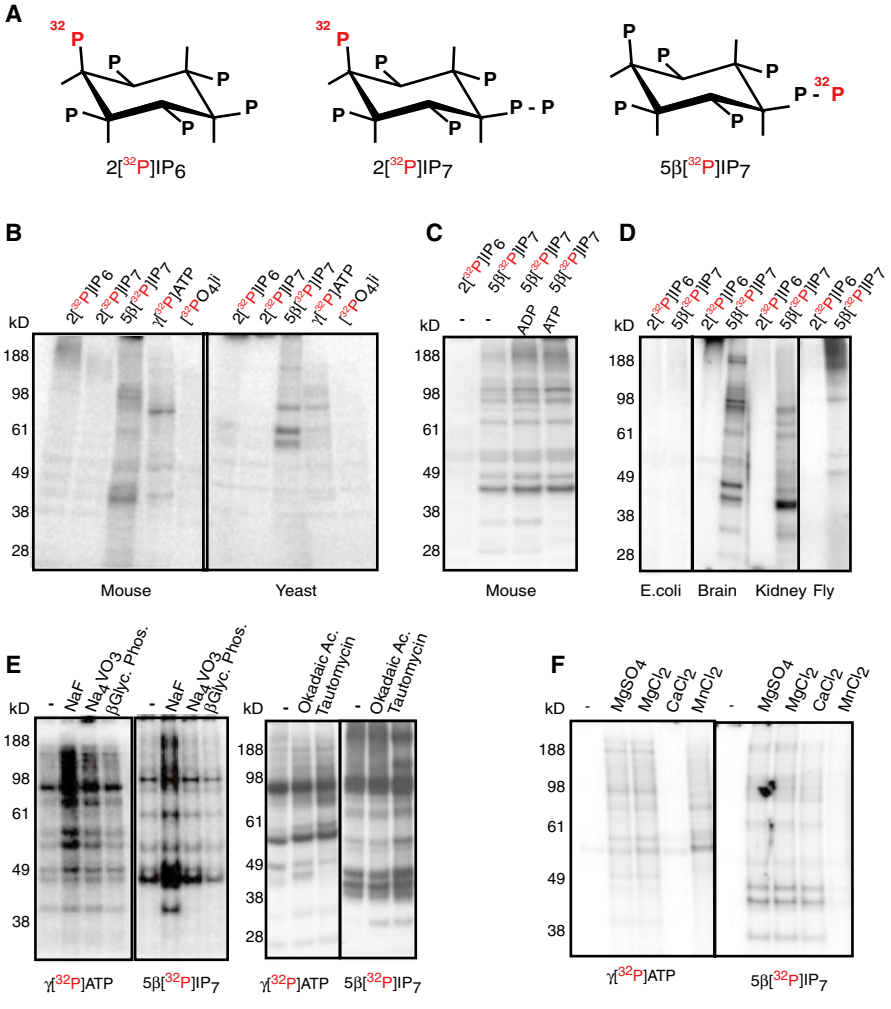

Fig. 1. Comparison of $\gamma[^{32}P]ATP$ and $5\beta[^{32}P]IP_7$ mediated protein phosphorylation. (**A**) Molecular structures of radiolabeled $2[^{32}P]IP_6$, $2[^{32}P]IP_7$, and $5\beta[^{32}P]IP_7$. (**B**) IP_7 and ATP elicit phosphorylation in eukaryotic tissue. Crude extracts from mouse brain or yeast (20 μg), prepared without any protease or phosphatase inhibitors, were incubated with 0.1 μCi of 30 Ci/mmol $2[^{32}P]IP_6$, $2[^{32}P]IP_7$, $5\beta[^{32}P]IP_7$, $\gamma[^{32}P]ATP$, or $[^{32}PO_4]_i$ for 1 hour at 37°C, followed by incubation in the presence of sample buffer at 95°C. Proteins were separated by polyacrylamide gel electrophoresis with NuPAGE (Novex), and the radiolabeled proteins were visualized by autoradiography. (**C**) Excess ADP or ATP fail to alter IP_7 phosphorylation. Mouse brain extract (*26*) was incubated with 0.5 μCi of 600 Ci/mmol radiolabeled inositols for 20 min at 37°C in the presence or absence of 1 mM unlabeled ATP or ADP. The samples were processed and visualized as above. (**D**) IP_7 phosphorylation in diverse species. Extracts from different organisms and tissues were incubated with labeled inositols as described in panel (C). (**E**) Protein phosphatase inhibitors augment IP_7 phosphorylation. Protein phosphorylation was performed as described in (C) in the presence or absence of the following protein phosphatase inhibitors: 0.5 mM NaF, 100 μM Na_3VO_4, 1 mM β-glycerol phosphate, 0.1 μM okadaic acid, and 0.1 μM tautomycin. (**F**) IP_7 phosphorylation requires divalent cations. Protein phosphorylation was performed as described in (C) by using 5 mM of the indicated bivalent cation as a cofactor.

ogy search of the yeast protein database revealed a substantial acidic Ser region in SRP40 (ORF YKR092c), a nucleolar protein that functions as a ribosomal chaperone (*14*). SRP40 overexpressed in yeast was also phosphorylated by 5β[^{32}P]IP_7 (Fig. 2C). SRP40 is the 63-kD target of 5β[^{32}P]IP_7 phosphorylation in yeast extracts on the basis of its loss of phosphorylation in extracts derived from SRP40-null (*srp40*Δ) yeast (Fig. 2E). Similarly, deletion of *nsr1* resulted in a loss of phosphorylation of the 60-kD protein. Because NSR1 and SRP40 are the principal proteins phosphorylated by 5β[^{32}P]IP_7, they could consume a major portion of endogenous IP_7. Hence, yeasts that do not have NSR1 or SRP40 (whose endogenous levels are only 15% those of NSR1) (*15*) might exhibit elevated levels of endogenous IP_7 and IP_8. IP_7 and IP_8 levels were doubled in mutant yeast lacking NSR1 compared with wild-type yeast but were unchanged in yeast lacking SRP40 (Fig. 2F). However, we cannot exclude that secondary consequences of *nsr1* deletion could indirectly alter IP_7 levels.

Although NSR1 consumes IP_7 within cells, it does not act as an IP_7 phosphatase (fig. S3). There was no detectable release of $[^{32}PO_4]_i$ orthophosphate in the presence of SRP40, YGR130c, or NSR1 under conditions in which these proteins were phosphorylated by 5β[^{32}P]IP_7. In contrast, the yeast IP_7 phosphatase, diphosphoinositol polyphosphate phosphohydrolase (DIPP, ORF YOR163w) (*16*), rapidly released $[^{32}PO_4]_i$ orthophosphate from 5β[^{32}P]IP_7 and did not undergo phosphorylation (fig. S3). These data rule out the possibility that apparent phosphorylation of proteins by IP_7 reflects a role for them as intermediates in a phosphohydrolase reaction.

To determine whether phosphorylation by IP_7 uses protein kinases, we examined phosphoproteins in the 89 viable null mutant yeast strains lacking each of the 122 known protein kinases (*17*). No striking alterations in the 5β[^{32}P]IP_7 protein phosphorylation pattern were observed in any of these mutants (*18*). In an in-gel kinase assay with purified NSR1, phosphorylation with 5β[^{32}P]IP_7 but not 2[^{32}P]IP_6 was observed, which ruled out the involvement of any protein kinase other than one that would have a molecular weight identical to NSR1 (Fig. 3A).

Heating increased the rate and extent of phosphorylation of substrates by 5β[^{32}P]IP_7, which further indicated that phosphorylation is nonenzymatic (Fig. 3B). There is no incorporation of [^{32}P]IP_7 labeled at position 2 nor of [^{32}P]IP_6 into purified SRP40 (Fig. 3C). Because the initial binding of IP_7 to the site of phosphorylation should require physiologic temperatures to ensure the appropriate conformation of the phosphorylation site, substantial phosphorylation of SRP40 by 5β[^{32}P]IP_7 was observed when conditions were first at 25°C followed by a 95°C treatment. No phosphorylation occurred if 5β[^{32}P]IP_7 was added after denaturing the proteins at 95°C or in presence of a chemical denaturing agent such as urea (Fig. 3D). Time-dependent phosphorylation of SRP40 and NSR1 at 37°C was also observed (Fig. 3E; fig. S4). Furthermore, 5β[^{32}P]IP_7-mediated phosphorylation of purified protein was detected in the presence of 50-fold excess of unlabeled IP_6, conditions that mimic the IP_6/IP_7 physiological ratio (fig. S4). Using multiple concentrations of unlabeled IP_7 (*18*), we determined that the K_m for IP_7-mediated protein phosphorylation was about 0.7 μM and the V_{max} was 0.1 μmol/mg per min. Although we detected 5β[^{32}P]IP_7-mediated phosphorylation of a fusion protein containing glutathione *S*-transferase and amino acid region 51 to 166 of NSR1 (Fig. 2D), no binding of [^{3}H]IP_6 or [^{3}H]IP_7 to this protein was detected (*18*). However, low affinity binding may have escaped detection.

To further characterize phosphorylation by IP_7, proteins phosphorylated by 5β[^{32}P]IP_7 were treated with acid or alkali. Phosphorylated YGR130c, SRP40, and NSR1 were more sensitive to alkali than to acid treatment (Fig. 3F), indicative of Ser/Thr phosphorylation (*19*). Furthermore, Western blot analysis of 5β[^{32}P]IP_7-phosphorylated SRP40 and NSR1 proteins with an antibody against phosphoThr revealed no phosphorylation on Thr (*18*), which suggested that phosphorylation of these proteins occurred on serine residues. SRP40 and NSR1 have very long stretches of serine residues that preclude precise mapping by site-directed mutagenesis. However, Nopp140, the mammalian homolog of SRP40 (*14*), has several short serine stretches surrounded by acidic amino acids. 5β[^{32}P]IP_7 phosphorylated both Nopp140 and Treacher Collins–Franceschetti syndrome 1 (TCOF1) protein, another mammalian nucleolar protein having short acidic serine stretches (*20*, *21*) (fig. S5).

Phosphorylation of regions consisting of amino acids 1 to 241, 1 to 100, and 1 to 75 of

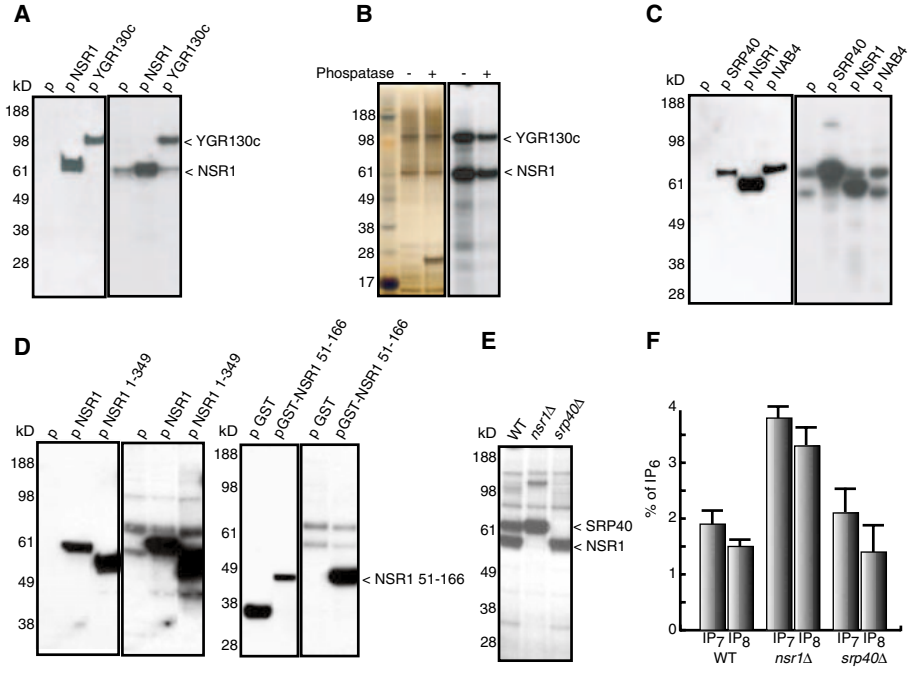

Fig. 2. Identification of 5β[^{32}P]IP_7 phosphorylation targets. (**A**) IP_7 phosphorylates NSR1 and YGR130c. Crude extracts from yeast overexpressing NSR1 or YGR130c (20 μg) were incubated with 0.5 μCi of 600 Ci/mmol 5β[^{32}P]IP_7 for 30 min at 37°C, followed by incubation at 95°C for 5 min in the presence of sample buffer. Samples were then separated by NuPAGE and transferred onto a nitrocellulose membrane. An autoradiogram of the membrane was obtained in order to visualize radiolabeled proteins (right) and subsequently analyzed by Western blot for Xpress tag (left) to assess protein expression. (**B**) Lambda phosphatase diminishes NSR1 and YGR130c phosphorylation. Purified NSR1 and YGR130c were incubated with 5β[^{32}P]IP_7 as described (*26*), in the presence (+) or absence (–) of lambda phosphatase (40 U). The gel was subjected to silver staining (left) and autoradiography (right). (**C** and **D**) Identifying consensus sequences for IP_7 phosphorylation. Extracts from yeast expressing full-length NSR1, NAB4, and SRP40 (C), or NSR1 fragments fused to an Xpress tag or to GST (D) were incubated with 5β[^{32}P]IP_7 and processed as described in (A). (**E**) SRP40 is a target of IP_7 phosphorylation. Protein extracts of wild-type, *nsr1*Δ, and *srp40*Δ yeast were incubated with 5β[^{32}P]IP_7 and processed as described in (A). (**F**) Inositol pyrophosphate levels are augmented by deletion of *nsr1*. Analysis of the intracellular concentration of inositol pyrophosphates in wild-type, *nsr1*Δ, and *srp40*Δ yeast. The concentrations of the inositol pyrophosphates IP_7 and IP_8 are expressed as a percentage of their ratio to IP_6 concentration. Data represent the means and SEM of three independent determinations. Concentrations of inositol phosphates were determined as described (*26*).

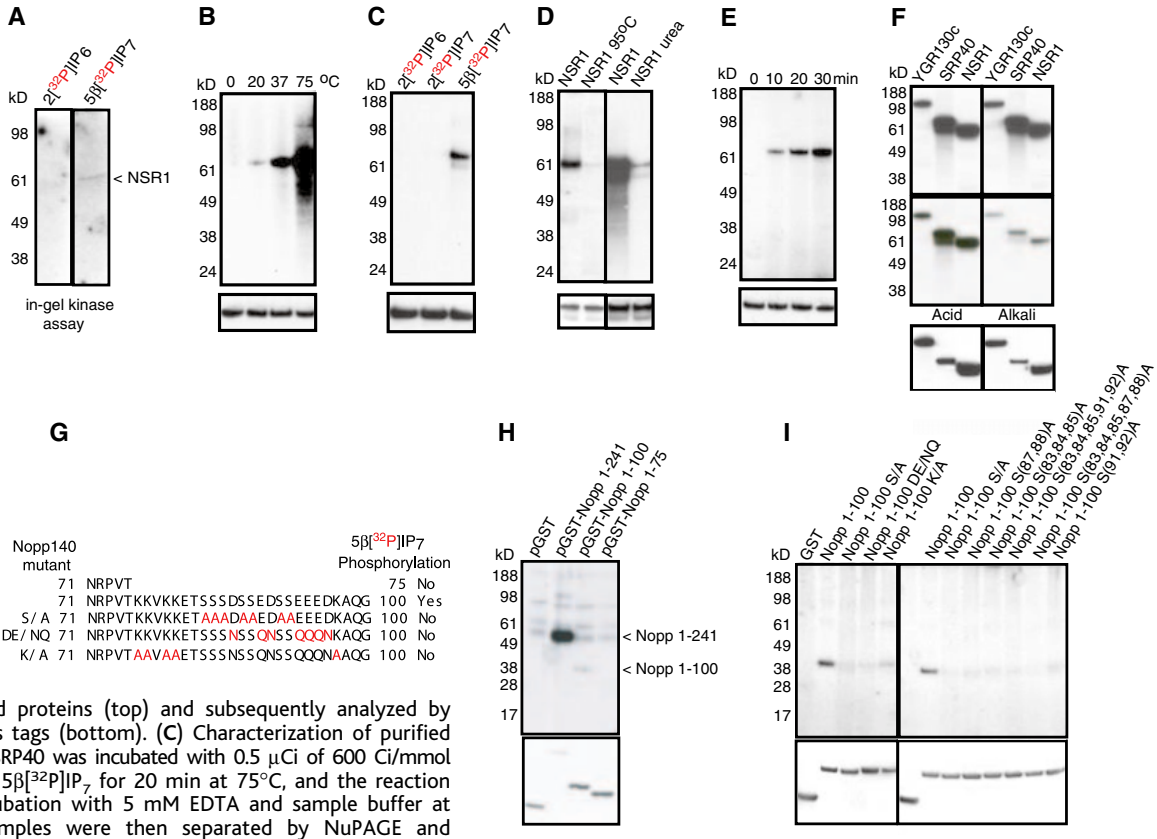

Fig. 3. Characterization of 5β[^{32}P]IP$_7$ phosphorylation. (**A**) 5β[^{32}P]IP$_7$ -driven protein phosphorylation does not require kinases. Autoradiograms of purified NSR1 subjected to denaturing SDS-PAGE and in-gel kinase assay (*26*). (**B**) Temperature-dependent phosphorylation of purified SRP40. SRP40 was incubated with 5β[^{32}P]IP$_7$ for 10 min. Phosphorylation assays were performed at the indicated temperatures, and sample buffer was added at room temperature. Samples were then separated by NuPAGE and transferred onto a nitrocellulose membrane. An autoradiogram of the membrane was obtained in order to visualize radiolabeled proteins (top) and subsequently analyzed by Western blot for Xpress tags (bottom). (**C**) Characterization of purified SRP40 phosphorylation. SRP40 was incubated with 0.5 μCi of 600 Ci/mmol 2[^{32}P]IP$_6$, 2[^{32}P]IP$_7$, and 5β[^{32}P]IP$_7$ for 20 min at 75°C, and the reaction was terminated by incubation with 5 mM EDTA and sample buffer at room temperature. Samples were then separated by NuPAGE and transferred onto a nitrocellulose membrane and processed as in (B). (**D**) Denatured NSR1 is not phosphorylated by 5β[^{32}P]IP$_7$. Purified, native NSR1 (at room temperature), heat-denatured NSR1 (at 95°C), or chemically denatured NSR1 (in 6 M urea), were incubated for 10 min with 5β[^{32}P]IP$_7$. Reactions were then incubated for 5 min at 95°C, separated by NuPAGE, and processed as in (B). (**E**) Time course of IP$_7$ phosphorylation of purified SRP40. SRP40 was incubated with 0.5 μCi of 600 Ci/mmol of 5β[^{32}P]IP$_7$ at 37°C for the indicated length of time. The reactions were terminated by adding 5 mM EDTA and sample buffer at room temperature; the samples were separated by NuPAGE and processed as in (B). (**F**) Alkali sensitivity of 5β[^{32}P]IP$_7$ phosphorylated proteins. YGR130c, SRP40, and NSR1 (20 to 50 ng) phosphorylated by 5β[^{32}P]IP$_7$ were separated by NuPAGE and transferred onto a PVDF membrane. An autoradiogram of the membrane was obtained in order to visualize radiolabeled proteins (top). Subsequently the membranes were subject to alkali or acid treatment (*26*), followed by a second autoradiogram (center). Xpress-tag Western blotting (bottom) was then performed to assess protein presence. (**G**) Amino acid sequences of the regions between residues 70 and 100 of mouse Nopp140 phosphorylated by 5β[^{32}P]IP$_7$. Mutations are highlighted in red, and the results of in vitro phosphorylation of these mutants by 5β[^{32}P]IP$_7$ are indicated. (**H**) Identification of IP$_7$ phosphorylation domain in Nopp140. Crude extracts (20 μg) from yeast overexpressing mouse Nopp140 fragments fused to GST were incubated with 0.5 μCi of 600 Ci/mmol 5β[^{32}P]IP$_7$ for 20 min at 37°C, followed by incubation at 95°C for 5 min in the presence of sample buffer. Samples were then separated by NuPAGE and transferred onto a nitrocellulose membrane. An autoradiogram of the membrane was obtained in order to visualize radiolabeled proteins (top) and subsequently subjected to GST antibody tag Western blotting (bottom) to assess protein expression. (**I**) Identification of the residues required for IP$_7$ phosphorylation. Purified Nopp140 fragments (1 μg) expressed as GST fusion proteins were incubated with 1 μCi of 60 Ci/mmol 5β[^{32}P]IP$_7$ for 15 min at 37°C and then in sample buffer at 95°C for 5 min. The proteins were separated by NuPAGE polyacrylamide gel electrophoresis (Novex), stained by Coomassie brilliant blue R-250 (bottom), and subject to autoradiography (top).

Nopp140 was assessed in the context of glutathione *S*-transferase (GST) fusion proteins (Fig. 3H). The most robust phosphorylation occurred with region 1 to 241, less with 1 to 100, and none was detected in region 1 to 75, which indicated that the seven Ser residues in Nopp140 between amino acids 75 and 100 are possible targets of phosphorylation (Fig. 3, G and H). Mutagenesis of these Ser residues to Ala abrogated phosphorylation by 5β[^{32}P]IP$_7$ (Fig. 3I). Because phosphorylation of the 1– to 241–amino acid fragment was greater than the 1– to 100–amino acid fragment, we assume that other acidic Ser residues within the 127 to 136 and 170 to 179 amino acid sequences are also potential phosphorylation targets. To map the exact phosphorylated Ser residues, we made combinatorial substitutions of two or more Ser with Ala within the 1– to 100–amino acid region of Nopp140 (Fig. 3I). Each mutant showed a decrease in phosphorylation, suggesting that multiple serines are phosphorylated, although it is conceivable that multiple serine mutations affect phosphorylation by altering the protein's secondary structure. The acidic amino acids that flank the serine stretches are critical, as their mutation resulted in loss of phosphorylation (Fig. 3I). The regions of phosphorylation by IP$_7$ also contain multiple lysines, the mutation of which dramatically decreased phosphorylation (Fig. 3I). The acidic amino acids are presumably required for binding to magnesium, whereas the lysine residues may coordinate the phosphate groups of IP$_7$. The failure of lysine mutations to abolish phosphorylation indicates that the lysines may not be absolutely critical.

To establish that IP$_7$ phosphorylation occurs with endogenous IP$_7$ and endogenous substrate proteins in intact cells, we focused on NSR1. NSR1 phosphorylation in vitro increased in extracts from yeast lacking IP6K (also know as KCS1, ORF YDR017c), which suggested that a major portion of endogenous NSR1 is phosphorylated under basal conditions by IP$_7$ (fig. S6). Endogenous IP$_7$ was radiolabeled by incubating yeast with [^{32}PO$_4$]$_i$ orthophosphate. Because IP6K plays a role in phosphate uptake (*22*, *23*), phosphate accumulation was reduced in yeast lacking IP6K (*ip6kΔ*) (fig. S7). To overcome the difficulties associated with overexpression, we used TAP (tandem affinity purification) yeast strains (*15*)

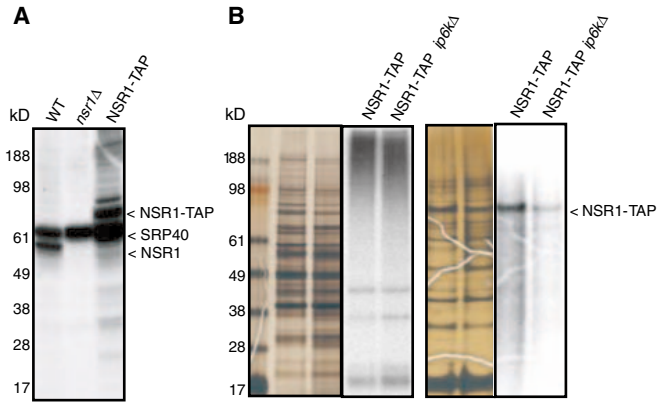

Fig. 4. In vivo 5β[^{32}P]IP$_7$ phosphorylation. (**A**) Presence of an 80-kD phosphorylated band in the NSR1-TAP strain. Wild-type, *nsr1*Δ, and wild-type NSR1-TAP yeast extracts were incubated with 5β[^{32}P]IP$_7$ and processed as described in Fig. 2. (**B** and **C**) IP6K deletion reduces phosphorylation of NSR1 by endogenous IP$_7$. (B) Wild-type NSR1-TAP and NSR1-TAP *ip6k*Δ yeast were labeled with [$^{32}PO_4$]$_i$ orthophosphate, and TAP-NSR1 was purified as described (*26*). Yeast homogenates (2 μg) were subjected to NuPAGE, and the gel was silver stained and autoradiographed to demonstrate equal levels of basal phosphorylation (left). Silver staining and autoradiogram of purified NSR1-TAP from wild-type and *ip6k*Δ yeast (right). (C) Quantification of the relative phosphorylation of NSR1-TAP purified from wild-type or *ip6k*Δ yeast. Data represent the mean values and SEM from three independent experiments.

to purify endogenous [$^{32}PO_4$]-labeled NSR1-TAP from both wild-type NSR1-TAP and *ip6k*Δ NSR1-TAP yeast (Fig. 4A). Lack of IP6K resulted in an almost 60% decline in phosphorylation of NSR1 in vivo, indicating that this protein is physiologically phosphorylated in intact cells by endogenous IP$_7$ (Fig. 4, B and C).

This study establishes that the inositol pyrophosphate IP$_7$ is a physiologic phosphate donor to a range of proteins in eukaryotic cells. The proteins we have best characterized as IP$_7$ targets, yeast NSR1 and SRP40 and mammalian Nopp140 and TCOF1, are nucleolar proteins involved in ribosomal biogenesis. Additionally, IP$_7$ phosphorylation of proteins involved in endocytosis may mediate roles of inositol pyrophosphates in this process (*6*), consistent with the phosphorylation by IP$_7$ of the adaptin β3A subunit (*18*), a regulator of vesicular trafficking (*24*, *25*).

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26. Materials and methods are available as supporting material on *Science* online.

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Materials and Methods
Figs. S1 to S7
References and Notes

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Nutrient Availability Regulates SIRT1 Through a Forkhead-Dependent Pathway

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Nutrient availability regulates life-span in a wide range of organisms. We demonstrate that in mammalian cells, acute nutrient withdrawal simultaneously augments expression of the SIRT1 deacetylase and activates the Forkhead transcription factor Foxo3a. Knockdown of Foxo3a expression inhibited the starvation-induced increase in SIRT1 expression. Stimulation of SIRT1 transcription by Foxo3a was mediated through two p53 binding sites present in the SIRT1 promoter, and a nutrient-sensitive physical interaction was observed between Foxo3a and p53. SIRT1 expression was not induced in starved p53-deficient mice. Thus, in mammalian cells, p53, Foxo3a, and SIRT1, three proteins separately implicated in aging, constitute a nutrient-sensing pathway.

In the yeast *Saccharomyces cerevisiae* and in the nematode *Caenorhabditis elegans*, lifespan can be extended by increasing the expression of the deacetylase Sir2, an enzyme whose activity depends on the oxidized form of nicotinamide adenine dinucleotide (NAD) (*1*, *2*). In these model organisms, the ability of Sir2 to extend life may be related to its role in gene silencing. In both the nematode and yeast, certain simple environmental stresses can also increase life-span. In yeast, reducing the amount of available glucose has this effect. The ability of glucose restriction to increase the life-span of yeast requires Sir2 (*3*). In *C. elegans*, activation of the Forkhead transcription factor DAF-16 is also associated with increased life-span (*4*) and its activation depends in part on nutrient availability (*5*). Genetic evidence further suggests that in worms, DAF-16 and Sir2 work through a common pathway (*2*), and recent evidence suggests that their mammalian counterparts physically interact (*6*, *7*).

Here, we further analyzed the interrelationship of the closest mammalian orthologs

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of DAF-16 and Sir2: the Forkhead transcription factor Foxo3a and the mammalian NAD-dependent deacetylase SIRT1. Using a mammalian model of nutritional stress (*8*), we examined the effects of nutritional withdrawal on the activity of the SIRT1 promoter. When a mammalian cell line (PC12) was starved overnight of both serum and glucose, SIRT1 promoter activity increased by a factor of ~4 relative to cells maintained in complete medium (Fig. 1A). Levels of *SIRT1* mRNA (Fig. 1B) (fig. S1) and protein expression (Fig. 1C) also increased under these conditions. Under normal nutrient conditions, a fusion protein comprising Foxo3a and green fluorescent protein (GFP) was primarily cytosolic (Fig. 1D). However, within 1 hour after nutrient withdrawal, the fusion protein was predominantly found within the nucleus (Fig. 1D) (fig. S2). Starvation also increased Foxo3a protein expression (fig. S3). The activity of a Forkhead-dependent luciferase reporter increased under starved conditions in two different mammalian cell lines (PC12 cells, Fig. 1E; HeLa cells, fig. S4). Starvation also induced the expression of a subset of other previously identified Forkhead transcriptional targets (fig. S5). When mice were subjected to an overnight fast, *SIRT1* mRNA increased in numerous tissues including skeletal muscle and liver (Fig. 1F) (fig. S6). These results suggest that in mammalian cells, nutritional stress induces both SIRT1 transcription and Foxo3a activation.

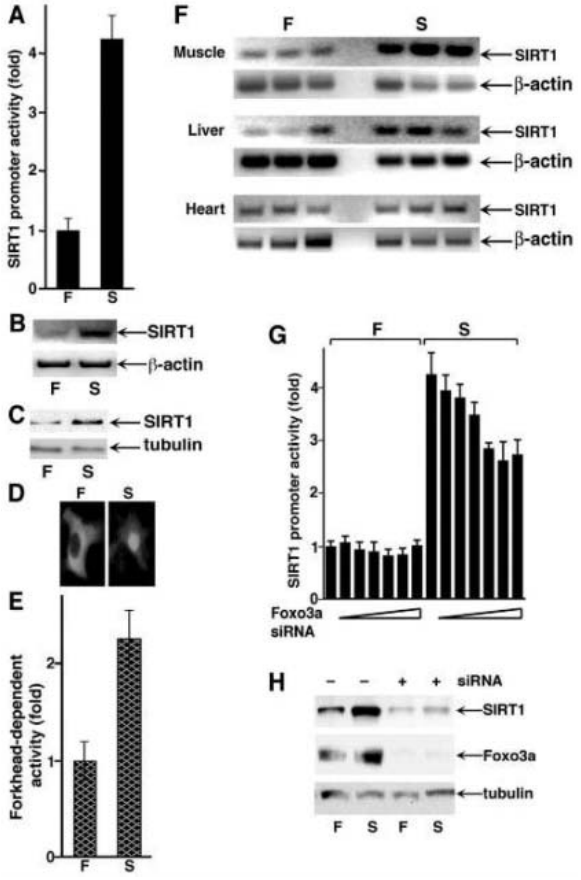

Fig. 1. SIRT1 induction by nutritional stress requires Foxo3a. (**A**) Normalized luciferase activity under the control of a ~1.5-kb fragment of the SIRT1 promoter in PC12 cells under either normal nutrient (fed, F) or starved (S) conditions. (**B**) Reverse transcription polymerase chain reaction (RT-PCR) analysis of *SIRT1* mRNA isolated from PC12 cells under fed or starved conditions. β-actin transcript was used as a loading control. (**C**) Western blot analysis of SIRT1 expression in PC12 cells under fed or starved conditions, with tubulin as a loading control. (**D**) Distribution of a Foxo3a-GFP fusion protein in PC12 cells under fed conditions or 1 hour after nutrient withdrawal (magnification 400×). (**E**) Luciferase reporter construct under the control of a synthetic Forkhead promoter in PC12 cells under fed conditions (F) or after 15 hours of starvation (S). (**F**) RT-PCR analysis of *SIRT1* mRNA from mice fed ad libitum or subjected to an overnight fast, with β-actin transcript as a loading control (n = 3 per group). (**G**) SIRT1 promoter activity in PC12 cells after transfection with increasing amounts of a siRNA plasmid directed against Foxo3a under either fed (F) or starved (S) conditions. (**H**) Western blot analysis of SIRT1 and Foxo3a expression, under fed (F) or starved (S) conditions, in two clonal PC12 cell lines, one with antibiotic resistance alone (–) and one with both antibiotic resistance and stable expression of *Foxo3a* siRNA (+). Tubulin expression was used as a loading control. Error bars are ±SD of triplicate determinations.

To determine whether Forkhead activity is required for the observed induction of SIRT1 expression under starved conditions, we used small interfering RNA (siRNA) to inhibit endogenous Foxo3a expression. Transient expression of a *Foxo3a* siRNA reduced SIRT1 promoter activity under starved conditions (Fig. 1G). In a stable cell line expressing *Foxo3a* siRNA constitutively, endogenous Foxo3a was greatly reduced (Fig. 1H). Similarly, whereas the control cell line exhibited a doubling of SIRT1 protein expression under starved conditions, cells with decreased Foxo3a expression had a reduced response.

Transient expression in PC12 cells of a constitutively active mutant of Foxo3a (Foxo3a-TM) stimulated the activity of a ~2.8-kb SIRT1 promoter fragment in the presence of nutrients (Fig. 2A). Successive deletions of the SIRT1 promoter revealed that the stimulatory effect of Foxo3a-TM was lost between positions –202 and –91. Lack of Forkhead binding sites in this region suggested that the stimulatory effect of Foxo3a-TM could be indirect. Interestingly, this region contains two consensus binding sites for the tumor suppressor protein p53 (Fig. 2B). To determine whether Foxo3a-TM might stimulate the SIRT1 promoter through these p53-binding motifs, we compared the stimulatory effects of Foxo3a-TM on the wild-type 202–base pair (bp) fragment, or with promoter fragments in which one or both of the p53-consensus binding sites were mutated. Mutation of either p53-binding site reduced the stimulatory effects of Foxo3a-TM (Fig. 2C). In the absence of both binding sites, Foxo3a-TM–dependent stimulation was reduced more then 90%. Furthermore, a synthetic promoter containing three tandem repeats of a 25-bp SIRT1 promoter fragment containing both p53 binding sites was activated by Foxo3a-TM to a degree that equaled or exceeded the observed effects of Forkhead proteins on the full-length SIRT1 promoter (Fig. 2D).

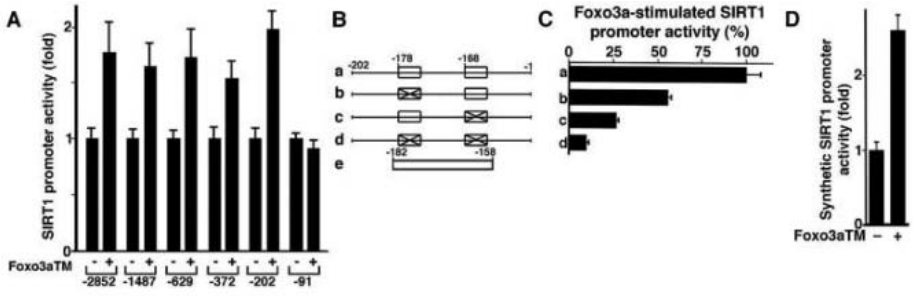

Fig. 2. The stimulatory effect of Foxo3a on SIRT1 transcription is mediated through two p53 binding sites. (**A**) Effect of Foxo3a-TM expression (+ or –) in PC12 cells on luciferase activity under the control of a series of SIRT1 promoter fragments. (**B**) Schematic diagram of a ~200-bp SIRT1 promoter fragment, showing the location of the two predicted p53 binding sites (hatched boxes). Constructs a to d represent fragments with neither, one, or both of these sites mutated. (**C**) Stimulatory effects of Foxo3a-TM expression on luciferase activity under the control of either the wild-type promoter or fragments shown in (B) containing p53 binding site mutations. (**D**) Foxo3a-TM stimulates luciferase activity under the control of a 3× tandem array of a 25-bp fragment (nucleotides –182 to –158, construct e) of the SIRT1 promoter. Error bars are ±SD of triplicate determinations.

These results raise the possibility that Foxo3a and p53 might physically and/or functionally interact. The Forkhead-associated (FHA) domain may mediate protein-protein interaction, and some FHA family members directly interact with p53 (*9*–*13*). Using purified recombinant proteins, we observed that p53 and Foxo3a appeared to directly interact in vitro (Fig. 3A). To localize the potential region of Foxo3a needed for p53 interaction, we generated a series of truncation mutants of Foxo3a in which successively more of the C terminus of the protein was deleted (Fig. 3B). Full-length and truncated mutants were expressed as GFP fusion proteins in PC12 cells to assess subcellular distribution. Under normal nutrient conditions, Foxo3a-TM was constitutively nuclear, whereas the distributions of the truncated Foxo3a mutants, $Foxo3a_{594}$ and $Foxo3a_{465}$, were cytosolic (similar to wild-type Foxo3a, Fig. 1D). In contrast, the location of the $Foxo3a_{361}$ truncation mutant was constitutively nuclear, presumably because of the lack of a nuclear export signal.

When overexpressed in HeLa cells, p53 immunoprecipitated with wild-type Foxo3a and each of the truncation mutants (Fig. 3C). The amount of associated p53 correlated better with the amount of nuclear, rather than total, Foxo3a. For instance, even though the overall expression level of Foxo3a-TM was considerably lower than that of wild-type Foxo3a, the amount of coimmunoprecipitated p53 was roughly similar. The amount of p53 after Foxo3a immunoprecipitation was greatest when the constitutively nuclear $Foxo3a_{361}$ mutant was expressed. This mutant also showed that the C terminus of Foxo3a is not required for interaction with p53. The interaction between Foxo3a and p53 was also observed when we performed reciprocal immunoprecipitation of p53 followed by Western blot analysis for Foxo3a (Fig. 3D).

Previous studies have identified a conserved histidine as essential for the interaction between certain FHA proteins and phosphopeptides (*9*, *10*). Nonetheless, mutagenesis of the corresponding histidine residue in Foxo3a did not affect binding to p53, as assessed by coimmunoprecipitation (fig. S7). However, the interaction between wild-type Foxo3a and p53 was strongly dependent on nutrient availability (Fig. 3E). This may simply reflect a difference in subcellular distribution of Foxo3a under normal nutrient or starved conditions (fig. S2); however, we cannot rule out the possibility that starvation-induced posttranslational modifications to either p53 or Foxo3a are important for their increased interaction.

p53 can function as either a transcriptional repressor or activator (*14*). Expression of p53 repressed the transcriptional activity of a reporter construct under the control of three tandem copies of a 25-bp element derived from the SIRT1 promoter (Fig. 3F). Coexpression of Foxo3a-TM relieved this inhibition. In contrast, p53 expression modestly activated transcription of an alternative synthetic p53 response element derived from the human ribosomal gene cluster. Coexpression of Foxo3a-TM inhibited p53-dependent transcriptional activity of this synthetic reporter (Fig. 3G). Thus, in PC12 cells, the physical interaction between Foxo3a and p53 antagonizes p53 function. Functional interaction between these two proteins was also observed in HeLa cells (fig. S8).

These in vitro results suggest a complex role for p53 in SIRT1 regulation. Under normal nutrient conditions, the predominant effect of p53 involves repression of SIRT1 (Fig. 3F). In contrast, under starved conditions, the ability of activated Foxo3a to stimulate SIRT1 expression requires p53 (Fig. 2C). These data suggest that in the absence of p53, the basal expression level of SIRT1 might rise but the starvation-induced increase would be blunted. To test this hypothesis, we analyzed SIRT1

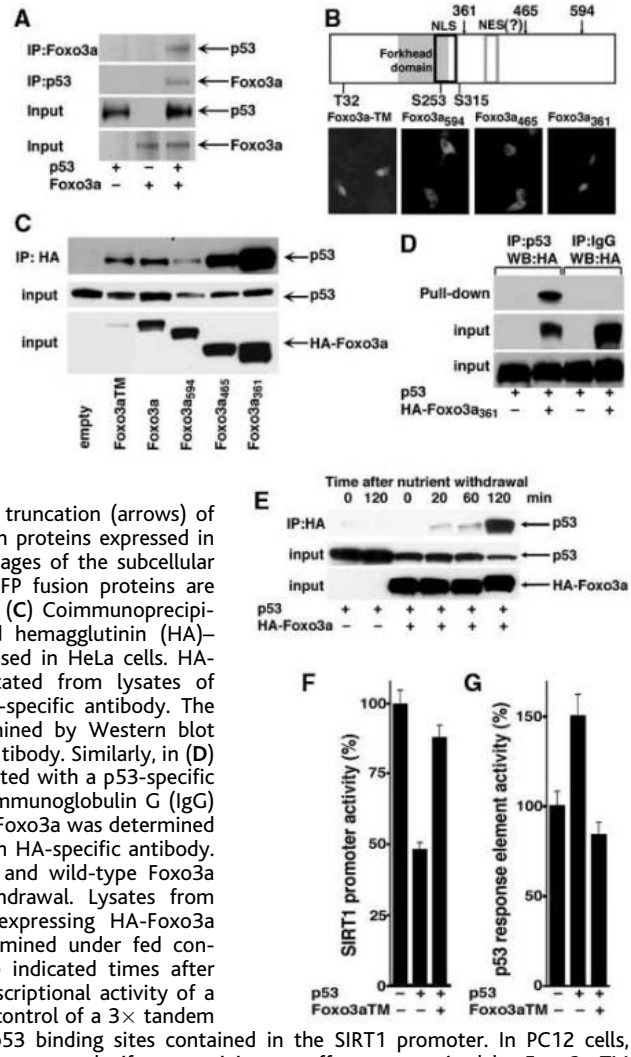

Fig. 3. Interaction between Foxo3a and p53. (**A**) In vitro interaction between wild-type p53 and Foxo3a. Recombinant proteins were incubated together, Foxo3a was immunoprecipitated (IP), and the presence of p53 was determined by Western blot analysis with a p53-specific antibody. Reciprocal immunoprecipitation of p53 followed by Foxo3a Western blot is also shown. (**B**) Schematic diagram of Foxo3a showing the nuclear localization sequence (NLS), nuclear export sequence (NES), the threonine (T) and serine (S) residues mutated in Foxo3a-TM, and the sites for truncation (arrows) of the various Foxo3a-GFP fusion proteins expressed in PC12 cells. Representative images of the subcellular distribution of the various GFP fusion proteins are shown (magnification 100×). (**C**) Coimmunoprecipitation of wild-type p53 and hemagglutinin (HA)–Foxo3a constructs overexpressed in HeLa cells. HA-Foxo3a was immunoprecipitated from lysates of transfected cells with an HA-specific antibody. The presence of p53 was determined by Western blot analysis with a p53-specific antibody. Similarly, in (**D**) lysates were immunoprecipitated with a p53-specific antibody or control isotype immunoglobulin G (IgG) sera, and the presence of HA-Foxo3a was determined by Western blot (WB) with an HA-specific antibody. (**E**) Interaction between p53 and wild-type Foxo3a increases after nutrient withdrawal. Lysates from transfected HeLa cells overexpressing HA-Foxo3a and wild-type p53 were examined under fed conditions (time = 0) or at the indicated times after nutrient withdrawal. (**F**) Transcriptional activity of a luciferase reporter under the control of a 3× tandem array containing the 25-bp p53 binding sites contained in the SIRT1 promoter. In PC12 cells, expression of wild-type p53 represses luciferase activity, an effect antagonized by Foxo3a-TM expression. (**G**) Transcriptional activity of another synthetic p53 response element. Error bars are ±SD of triplicate determinations.

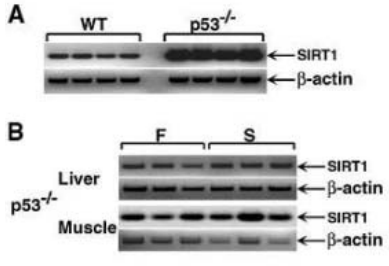

Fig. 4. Role of p53 in basal and starvation-induced SIRT1 expression. (**A**) RT-PCR analysis of basal SIRT1 expression in adipose tissue from wild-type (WT) or $p53^{-/-}$ mice. β-actin transcript was used as a loading control. (**B**) Lack of starvation-induced SIRT1 induction in $p53^{-/-}$ mice, as shown by RT-PCR analysis of *SIRT1* mRNA in liver and skeletal muscle of $p53^{-/-}$ animals under either fed (F; $n = 3$) or starved (S; $n = 3$) conditions. β-actin transcript was used as a loading control.

expression in mice with a targeted deletion in p53. Basal SIRT1 expression was higher in adipose tissue of $p53^{-/-}$ mice than in wild-type controls (Fig. 4A). This result is particularly interesting given the recent observation that SIRT1 plays a prominent role in fat usage (*15*). A survey of other tissues revealed a more modest but consistent increase in basal SIRT1 expression in the $p53^{-/-}$ mice (fig. S9). However, *SIRT1* mRNA did not appreciably change in either liver or skeletal muscle of $p53^{-/-}$ mice after overnight fasting (Figs. 1F and 4B). This lack of starvation-induced SIRT1 expression occurred even though, relative to wild-type animals, $p53^{-/-}$ mice had an even greater drop in their fasting glucose after food withdrawal (wild-type mice, 102 ± 7 mg/dl; $p53^{-/-}$ mice, 76 ± 8 mg/dl; $n = 4$ each, $P < 0.01$). These in vivo results further support a role for p53 in SIRT1 regulation.

Our results show that in mammalian cells, a simple model of acute nutritional stress results in a Foxo3a-dependent increase in SIRT1 levels. Interestingly, chronic caloric restriction also increases SIRT1 expression (*16*). Foxo3a regulation of SIRT1 expression occurs through an interaction with p53. In this regard, it is interesting to note that Forkhead proteins and p53 share a number of similarities (*14*, *17*). In the worm, Forkhead proteins respond to nutrient availability and a homolog of p53 regulates starvation response (*18*). In mammals, although p53 is often linked to cancer and Forkhead proteins are commonly associated with aging, recent evidence has suggested a role for Forkhead proteins in tumorigenesis (*19*, *20*) and a role for p53 in life-span (*21*, *22*). Finally, both Foxo3a and p53 directly and independently bind to SIRT1 (*6*, *7*, *23*, *24*). Taken together, these results suggest a complicated but undoubtedly important homeostatic regulatory network involving p53, Foxo3a, and SIRT1. Further analysis of this network may help us to understand how adaptation to certain cellular stresses, including nutrient availability, may modulate mammalian life-span.

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Cofolding Organizes Alfalfa Mosaic Virus RNA and Coat Protein for Replication

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Alfalfa mosaic virus genomic RNAs are infectious only when the viral coat protein binds to the RNA 3′ termini. The crystal structure of an alfalfa mosaic virus RNA-peptide complex reveals that conserved AUGC repeats and Pro-Thr-x-Arg-Ser-x-x-Tyr coat protein amino acids cofold upon interacting. Alternating AUGC residues have opposite orientation, and they base pair in different adjacent duplexes. Localized RNA backbone reversals stabilized by arginine-guanine interactions place the adenosines and guanines in reverse order in the duplex. The results suggest that a uniform, organized 3′ conformation, similar to that found on viral RNAs with transfer RNA–like ends, may be essential for replication.

A general problem in positive-strand RNA virology is understanding how viral RNA replication is initiated by the RNA-dependent RNA polymerase (replicase) on the correct template and nucleotide in an infected cell. Alfalfa mosaic virus (AMV) and ilarviruses are unusual positive-sense viruses, the genomic RNAs of which are replicated only in the presence of the viral coat protein (CP) (*1*, *2*). These viruses are distinguished from many other members of the virus family *Bromoviridae* because they lack canonical features of the tRNA-like structure (TLS) common at the 3′ termini of the viral RNA genomes. The TLS is a necessary and sufficient feature for recruitment of the bromovirus replicase (*3*, *4*). CP-induced structural organization of the AMV RNA 3′ terminus may create a functional homolog of the tRNA tail and thereby permit recognition by the RNA-dependent RNA polymerase.

CP binds specifically to the 3′ untranslated regions (3′UTRs) found on all four RNAs of the segmented AMV genome (*5*). The 180-nucleotide 3′UTR secondary structure likely consists of six hairpins, most of which are separated by single-stranded tetranucleotide AUGC repeats (*5*–*8*). These repeats are characteristic of AMV and ilarvirus RNA sequences and are important for CP binding (*8*–*11*). We previously identified a 39-nucleotide minimal high affinity AMV CP-binding site, consisting of the two terminal hairpins and their flanking AUGC nucleotides (nucleotides 843 to 881 in RNA4; i.e., $AMV_{843–881}$) (*8*, *12*, *13*) (fig. S1A). This fragment is competent to bind either full-length CP or a 26–amino acid peptide (CP26, fig. S1B) (*13*) representing the N-terminal RNA binding domain (*14*). The CP N terminus contains a Pro-Thr-x-Arg-Ser-x-x-Tyr (PTxRSxxY) RNA binding domain conserved among AMV and ilarvirus CPs (*14*). The arginine at position 17 is critical for both RNA binding and virus replication (*14*–*16*). Circular dichroism experiments suggest that the CP N terminus is unstructured in solution (*17*). Previous virus crystallization attempts required proteolytic cleavage of the AMV CP N terminus (*18*, *19*).

Crystals of the AMV N-terminal CP peptide CP26 in complex with 5-bromouridine–labeled $AMV_{843–881}$ RNA were grown in hanging drops by vapor diffusion. The structure was

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solved to 3.0 Å resolution with phases obtained by multiple-wavelength anomalous dispersion (table S1) (*13*). The cocrystal structure reveals that the RNA and peptides undergo cofolding events that substantially alter their structure from their unbound forms. The RNA forms two hairpins oriented at approximately right angles (Fig. 1A). Each of the RNA hairpins is extended by two base pairs formed between nucleotides from adjacent AUGC sequences (Figs. 1 and 2). Loop nucleotides could not be placed into the final model (fig. S2) (*13*). The two CP26 peptides each form an α helix with an extended N-terminal tail (Fig. 1A). Residues 12 to 26 are ordered in peptide 1, whereas residues 9 to 26 are ordered in peptide 2. The extreme N termini of both peptides remained flexible and were not visible.

A notable feature of the structure is that cofolding events promote base pairing between the AUGC repeats in the presence of CP. The U(844) and C(846) nucleotides pair with A(865) and G(867), respectively, at the base of duplex 1 (Figs. 1C and 2A). The C=G pair is a Watson-Crick pair, whereas the A-U pair is a noncanonical UA reverse Watson-Crick pair. An identical interaction is seen at the base of duplex 2, which is extended by two base pairs when U(866) and C(868) hydrogen bond with A(878) and G(880), respectively (Figs. 1C and 2B). The formation of these base pairs introduces two types of distortion into the AMV RNA. First, the interhelical $AUGC_{865–868}$ (purple in Figs. 1 and 2) participates in base pairing interactions in both duplex 1 and duplex 2. This requires alternating bases of $AUGC_{865–868}$ to be oriented in opposite directions. Second, to form C=G base pairs, G(867) and G(880) must be positioned upstream of A(865) and A(878) in their respective stacks, out of numerical order (Fig. 1B). The insertion of G(867) and G(880) between A(864) and A(865) or G(877) and A(878), respectively, introduces an unusual kink into the ribose-phosphate backbone (Fig. 1). In duplex 1, A(864) and A(865) are separated by the insertion of G(867) into the base stack. U(866) is flipped out of the base stack of duplex 1 and instead participates in reverse Watson-Crick base pairing at the base of duplex 2 (Fig. 2A). The same interaction is seen at the base of duplex 2 (Fig. 2B), where the distance between G(877) and A(878) is also increased to accommodate the insertion of G(880) between them to form a base pair with C(868).

Amino acids in the conserved RNA binding motif PTxRSxxY play key roles in stabilizing the AUGC base pairing interactions. Biochemical evidence suggesting the importance of arginine 17 (*14*) is consistent with its central position within the RNA protein-binding site. Both the G(867)-C(846) and G(880)-C(868) base pairs are displaced from the helical axis toward the minor groove, allowing the Arg^{17} sidechain guanidinium to insert into the base stack at the major groove. Arg^{17} is positioned to make polydentate hydrogen bonds with the G(867) (peptide 1) and G(880) (peptide 2) bases, thus extending the stacking interaction of the guanine bases with A(865) and A(878), respectively (Fig. 3A). The importance of arginine residues in RNA-peptide interactions was demonstrated previously in the human immunodeficiency virus Tat/Tar complex (*20*). The bottoms of both hairpins are capped by conserved tyrosine (Tyr^{21}) sidechains from peptides 1 and 2. Between Arg^{17} and Pro^{14}, both copies of the CP26 peptide transition from α helix to extended chain. This transition is mediated by a tight turn between Thr^{15} and Pro^{14}, forming an L shape that cradles the extended RNA duplex (Fig. 1A). The L turn allows the N-terminal tail of peptide 2 to straddle a groove in the RNA surface formed by duplex 1 and duplex 2, and to make additional contacts between the RNA backbone of duplex 1 and Lys^{13} and Gln^{19} of peptide 2 (Fig. 1B). This conformation is facilitated by the conserved Pro^{14} and is stabilized by an exchange of hydrogen bonds between the sidechain hydroxyls and the mainchain amides of Thr^{15} and Ser^{18} within the PTxRSxxY motifs (Fig. 3B). The side chain of Ser^{18} occupies a pocket that provides an optimal fit. It forms hydrogen

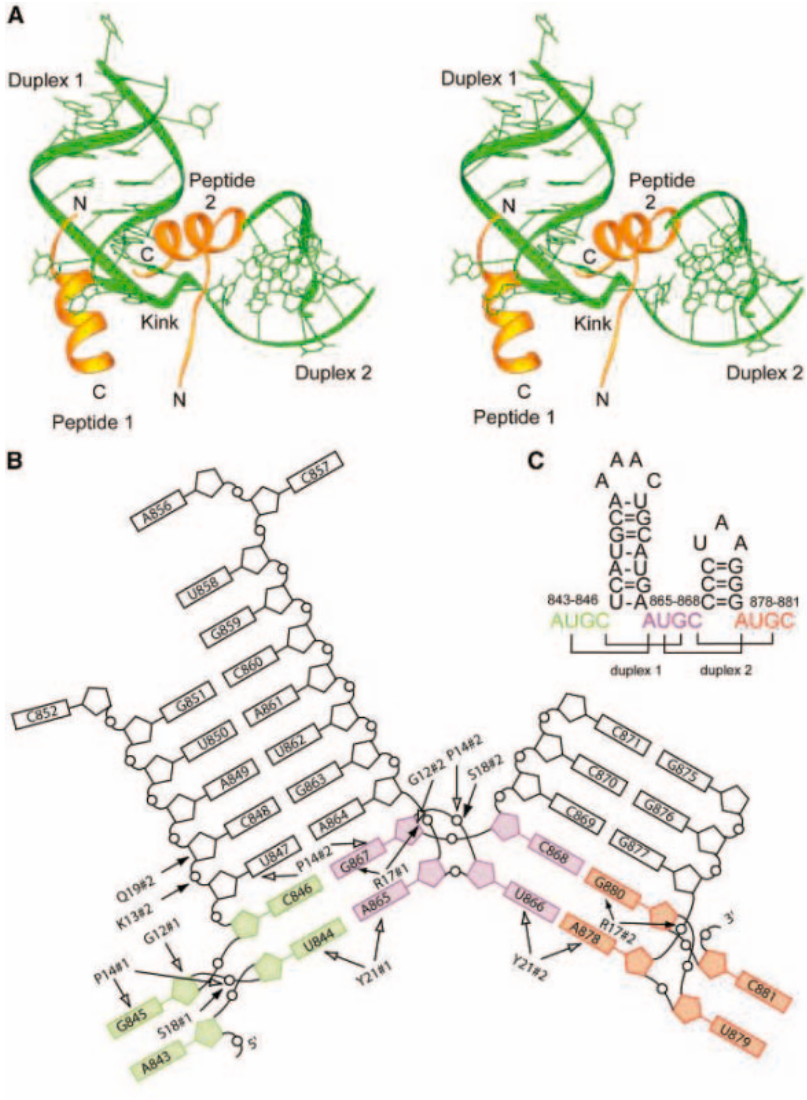

Fig. 1. (**A**) Stereoimage of the AMV RNA-CP complex. The RNA is shown in green and the CP26 peptides are shown in gold. (**B**) Schematic diagram of the AMV RNA and CP26 protein contacts. The AUGC sequences have been color coded. The 5′ AUGC is colored green, the interhelical AUGC sequence is colored purple, and the 3′ AUGC sequence is colored red. Hydrogen-bonding contacts between the RNA and peptide are indicated with filled arrows and van der Waals contacts are indicated by open arrows. #1, peptide 1; #2, peptide 2; G, Gly; K, Lys; P, Pro; Q, Gln; R, Arg; S, Ser; Y, Tyr. (**C**) Secondary structure of the 39-nucleotide minimal binding domain found at the 3′ end of AMV RNAs, $AMV_{843–881}$. The AUGC sequences are color coded as in (**B**). Base pairs are indicated by brackets.

bonds with the phosphate oxygen of G(845) (peptide 1) or G(867) (peptide 2) and the amide nitrogen of Thr[15] (Fig. 3B). Pro[14] makes van der Waals contacts with the ribose ring and phosphate oxygen of G(845) (peptide 1) and G(867) (peptide 2) (Fig. 1B). The edge of the Pro[14] sidechain makes additional favorable packing contacts with the ribose-phosphate backbone above and below the plane of the proline ring, contributing to the stability of the complex. Mutation of Pro[14], Ser[18], or Tyr[21] diminishes CP26-AMV$_{843–881}$ RNA binding affinity and replication activity (*21*), underscoring the importance of these contacts for the stability of the RNA-protein complex.

The structure correlates well with biochemical and functional data. Disruption of either the CP-binding site on the RNA or the RNA binding site on the CP prevents formation of the complex and blocks RNA replication (*12*, *15–17*, *22*). The contacts related to the L turn, which were not predicted by amino acid sequence homology, provide a structural basis for the observed protection of nucleotides 847 to 850 during hydroxyl radical footprinting experiments (*8*, *12*). In vitro RNA selection experiments (*10*, *11*) and hydroxyl radical footprinting data (*8*, *14*) suggested that peptides bind to the base of the hairpins without substantial loop interactions. The inter-AUGC base pairing contacts are consistent with previous chemical modification data and in vitro selection showing that modification of U(844) or C(846) diminished CP binding (*12*), whereas degenerate in vitro selection data show the strong conservation of nucleotide identity at the AUGC positions (*11*). The importance of the central AUGC and the dual hairpin contacts made in this region with peptide 2 (Fig. 1) was also anticipated on the basis of biochemical data revealing that peptide binding was abolished if tandem AUGC repeats separated the two hairpins (*23*).

The unusual inter-AUGC base pairing seen in the structure and the conservation of AUGC sequences among AMV and ilarvirus 3′UTRs (fig. S3A) prompted us to test whether CP can bind RNAs containing nucleotide substitutions that preserve base pairing, even though the substitutions are not strictly structurally equivalent. We changed all three AUGC sequences in AMV$_{843–881}$ to CGUA, GCAU, or UACG. None of these RNAs bound CP26 in mobility shift assays (fig. S3B) (*13*). The positions of G(867) and G(880) are likely to be critical for CP binding because of their close interaction with the critical Arg[17] residue. An additional UAGC mutant, designed to leave the position of the C=G base pair unchanged, also did not bind CP26. This observation is consistent with the proposal that the extended stacking of Arg[17]-G(867) and Arg[17]-G(880) with A(865) and A(878), respectively, contributes to the stability of the unusual base pair structure at the base of the stem loops.

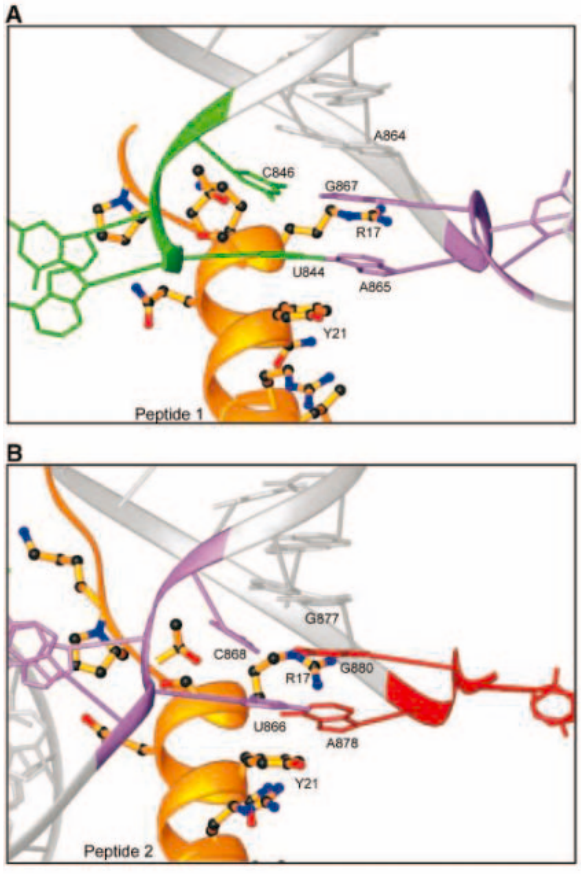

Fig. 2. Formation of inter-AUGC base pairs. (**A**) RNA-peptide interactions at the base of duplex 1. The CP peptide α helix and sidechains are shown in gold. The RNA is shown in gray. The 5′ AUGC sequence is colored green, and the interhelical AUGC sequence is colored purple. (**B**) Similar RNA-peptide interactions are seen at the base of duplex 2. The interhelical AUGC sequence is highlighted in purple and the 3′ AUGC sequence is colored red. R, Arg; Y, Tyr.

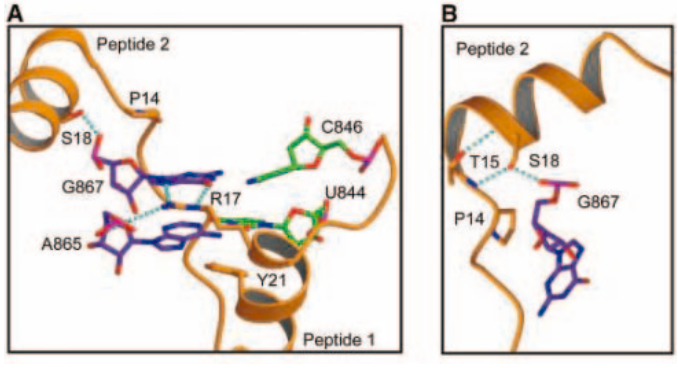

Fig. 3. Interactions between the AMV RNA and CP26 peptides. (**A**) View of the two additional base pairs at the base of duplex 1 and their interaction with peptide sidechains. The carbon atoms of base pairing members at the base of duplex 1 from the 3′ AUGC [U(844) and C(846)] are colored green, whereas members from the interhelical AUGC [A(865) and G(867)] are colored purple. Hydrogen bonds are indicated in cyan. (**B**) View of peptide 2 making the L-shaped turn. The carbon atoms of peptide 2 are colored gold, whereas those of G(867) are colored purple. P, Pro; R, Arg; S, Ser; T, Thr; Y, Tyr.

Features of the AMV RNA-CP peptide complex may shed light on the mechanisms at work during the initial phases of virus replication. The structure suggests that the RNA assumes a highly ordered conformation in the presence of CP. The AUGC regions, single stranded and potentially flexible in the absence of CP (*24*), form base pairs when bound to CP26 and force the RNA to assume a more conformationally limited form. The recurring pattern of peptide interactions with duplex 1 and duplex 2 RNAs (Figs. 1B and 2) suggests that these types of contacts may also occur at other U-C-hairpin-A-G motifs throughout the 3′UTR (*8*) (Fig. 4A). We modeled CP binding along the entire length of the 3′UTR by aligning the AUGC regions of multiple copies of the AMV$_{843–881}$-CP26 structure, while ignoring the differing lengths of the hairpins. The resulting molecule forms a long rodlike structure with the hairpins projecting out from a central axis (Fig. 4, B and C). A transition from a more flexible to a more constrained 3′ end upon CP binding agrees with a replication model suggesting that an RNA conformational change accompanies CP binding to the 3′ ends of the

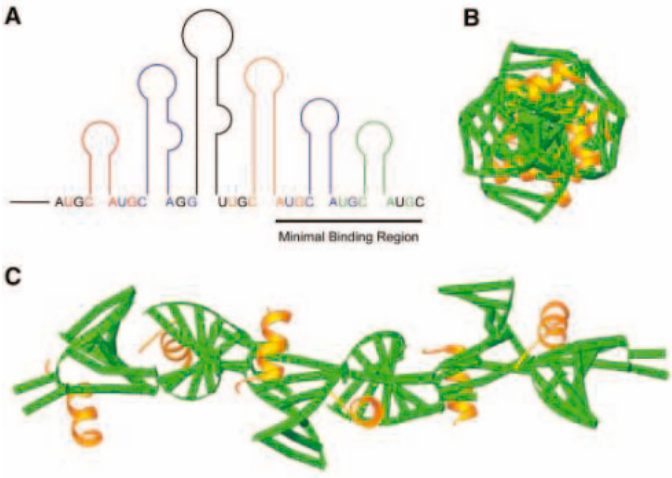

Fig. 4. Proposed structure of the entire 3′UTR. (**A**) Diagram of the 3′UTR with color-coded U-C-hairpin-A-G CP-binding sites. (**B**) End-on view of the simulated 3′UTR. (**C**) View of the 3′UTR along its length. RNA is colored green; peptide is colored yellow.

genomic RNAs (*25*). Circular dichroism analyses and polyacrylamide gel electrophoresis experiments also suggest that the AMV 3′UTR RNA becomes more compact in the presence of CP (*11*, *17*). Sequential loading of CP along the length of the 3′UTR may specify switches between replication, translation, and assembly processes. As a general theme in nonpolyadenylated positive-strand RNA viruses, the structural organization of the extreme 3′ terminus (here, by binding of two CP subunits at the base of the last two hairpins) may present a single conformation that is recognized by replicase enzymes. Organization of the entire length of the AMV 3′UTR (binding of CP molecules at the base of all hairpins) may specify assembly functions through the formation of a rodlike structure to nucleate bacilliform particle formation.

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Supporting Online Material

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bHLH Transcription Factor Olig1 Is Required to Repair Demyelinated Lesions in the CNS

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Olig1 and Olig2 are closely related basic helix-loop-helix (bHLH) transcription factors that are expressed in myelinating oligodendrocytes and their progenitor cells in the developing central nervous system (CNS). Olig2 is necessary for the specification of oligodendrocytes, but the biological functions of Olig1 during oligodendrocyte lineage development are poorly understood. We show here that Olig1 function in mice is required not to develop the brain but to repair it. Specifically, we demonstrate a genetic requirement for Olig1 in repairing the types of lesions that occur in patients with multiple sclerosis.

The oligodendrocyte is the myelinating cell of the central nervous system (CNS) (*1*, *2*). The appearance of these myelinating cells during evolution is thought to have enabled the vertebrate nervous system to grow large and complex by allowing saltatory conduction of nervous impulses (*3*). A variety of demyelinating diseases exist in humans, wherein the myelin sheaths are lost, usually through the death of mature oligodendrocytes. One principal example, multiple sclerosis (MS), affects roughly two and a half million people worldwide and is one of the most common causes of neurological disability in young adults (*4*–*6*). Typically, patients with MS present with a relapsing/remitting form of the disease, characterized by acute demyelinating episodes followed by the generation of new oligodendrocytes, remyelination, and functional recovery. However, remyelination is an inconsistent

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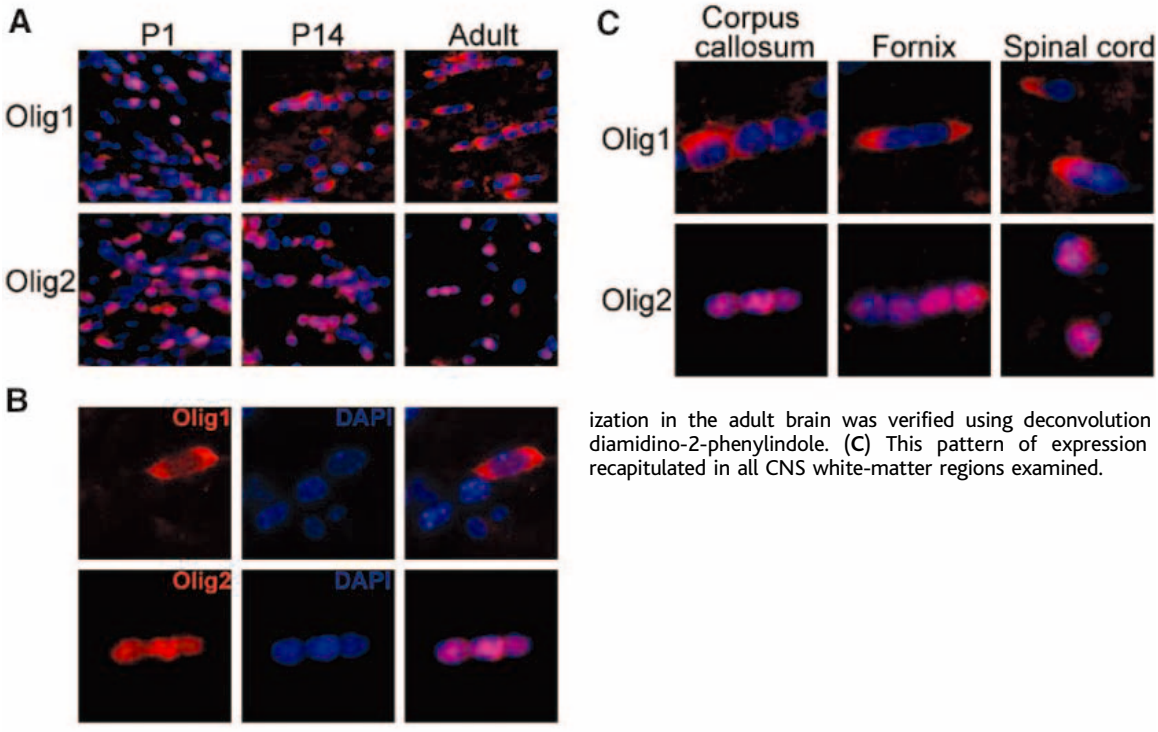

Fig. 1. Subcellular relocalization of Olig1, but not Olig2, during development. (**A**) Olig1 and Olig2 are both present in the nucleus of oligodendrocytes and their progenitors in the postnatal day 1 (P1) mouse brain. Olig2 continues to be nuclear at all developmental stages, whereas Olig1 is almost completely cytoplasmic in the adult mouse brain. (**B**) Subcellular localization in the adult brain was verified using deconvolution microscopy. DAPI, 4′,6′-diamidino-2-phenylindole. (**C**) This pattern of expression of Olig1 and Olig2 is recapitulated in all CNS white-matter regions examined.

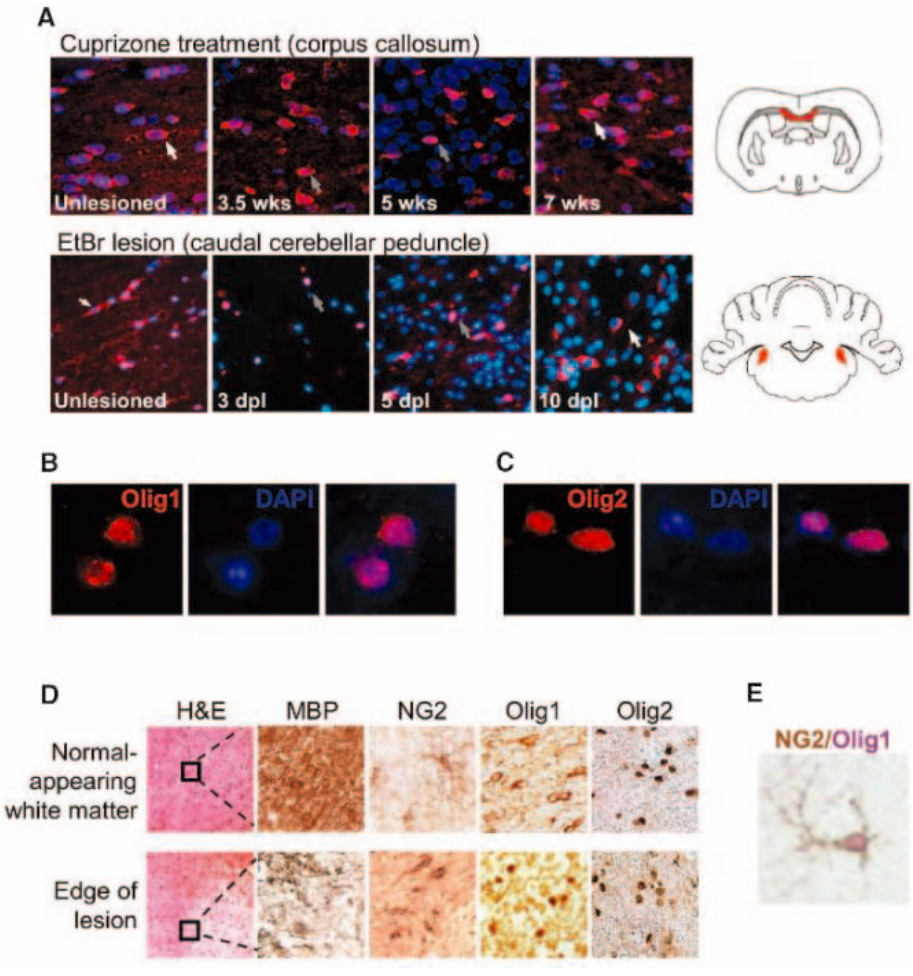

Fig. 2. Dynamic localization of Olig1 during remyelination. (**A**) Demyelination was induced in the brain with the dietary administration of cuprizone, and in the caudal cerebellar peduncle via an injection of ethidium bromide, and analyzed over various time points for cytoplasmic (white arrows) or nuclear (gray arrows) expression of Olig1. dpl, days post lesion. (**B** and **C**) Deconvolution microscopy was used to verify the nuclear localization of Olig1 and Olig2 during remyelination. (**D**) Postmortem tissue from six MS patients was examined for the subcellular localization of Olig1 and Olig2 surrounding demyelinated plaques. The examined tissue contained chronic demyelinated plaques, with varying degrees of inflammatory infiltrate, and varying degrees of remyelination occurring at the lesion edge. The images shown are serial sections from a single donor stained with hemotoxylin and eosin (H&E) or antibodies directed against mature oligodendrocytes and MBP, oligodendrocyte progenitors (NG2), Olig1, or Olig2. As indicated with black boxes in the H&E-stained sections, areas of normal-appearing white matter and a chronic active lesion were identified and maintained constant across the serial sections for imaging analysis. (**E**) Colocalization of NG2 (brown) and Olig1 (purple) in MS tissue demonstrated that Olig1 is nuclear in oligodendrocyte progenitors surrounding an MS lesion.

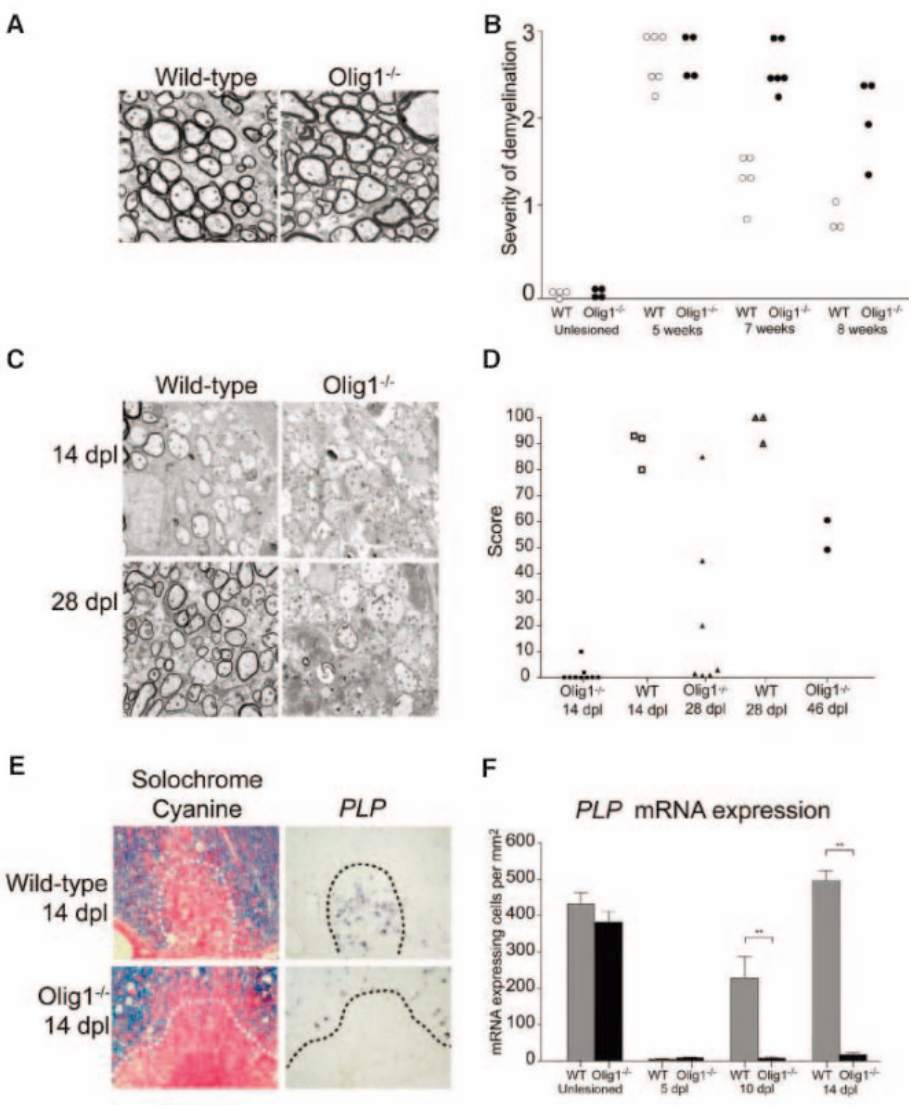

Fig. 3. Failure of remyelination in mice lacking Olig1. (**A**) Morphological analysis of myelinated tracts in the ventrolateral funiculus of the Olig1$^{-/-}$ mouse spinal cord indicates normal myelin formation and compaction. (**B**) Luxol fast blue stain and light microscopy show that Olig1 is required for efficient remyelination of the corpus callosum in the brains of wild-type (WT) and Olig1$^{-/-}$ mice after demyelination with cuprizone. Slides were scored in a double-blind fashion on a scale of zero to three, where a score of zero indicates complete myelination of all fibers at the midline corpus callosum, and a score of three indicates complete demyelination. (**C**) Olig1 is also required for efficient remyelination of lysolecithin-induced lesions in the dorsal or ventrolateral funiculi of the spinal cord. At 14 dpl, the WT mice exhibited extensive remyelination as seen by light microscopy (fig. S3A), with 80 to 90% of the axons within the lesion showing remyelination. The new myelin sheaths were very thin at this time, and so their presence was confirmed by means of electron microscopy (EM). This extensive remyelination contrasts dramatically with that demonstrated by the Olig1$^{-/-}$ mice at the same time point, where there was almost no evidence of remyelination as indicated by either light microscopy using standard morphological criteria (*29*) or by EM. (**D**) Quantitation of remyelination shown in (C). The extent of remyelination for each animal was assessed with light microscopy and each was given a score (by two independent experimenters) for the number of axons showing remyelination as a percentage of the total number of demyelinated axons. There is a significant difference at both 14 and 28 dpl in the extent of remyelination between WT (open symbols) and Olig1$^{-/-}$ mice (solid symbols), and remyelination in the Olig1$^{-/-}$ mice remains incomplete at 46 dpl (fig. S3, B and C). (**E** and **F**) Solochrome cyanine staining was used in all animals to determine the extent of the area of demyelination in the ventrolateral funiculus of the spinal cord. In normal unlesioned white-matter, the density of mature PLP/DM20-expressing oligodendrocytes is indistinguishable between WT and Olig1$^{-/-}$ mice. However, at 14 dpl the lesions in the WT mice contain differentiated oligodendrocytes, whereas there is an almost complete absence of mature oligodendrocytes within the Olig1$^{-/-}$ lesions at this time (*t* test, ** = $P < 0.0007$).

event in this disease, and the accumulated load of lesions that fail to remyelinate results in progressive neurological deterioration, in part because the capacity to generate new oligodendrocytes becomes limited (*7*–*11*).

To understand the molecular mechanisms of remyelination and its limitations, and thereby identify potential targets for therapeutic intervention to promote repair, it is instructive to look at the mechanisms that create oligodendrocytes in the developing CNS (*12*). The basic helix-loop-helix (bHLH) transcription factor Olig2 is necessary for specification of both oligodendrocytes and motor neurons during vertebrate embryogenesis (*13*–*15*). Olig2-null (Olig2$^{-/-}$) mice die at birth because of a deficit of motor neurons. A closely related homolog, Olig1, is coexpressed with Olig2 in many cells of the oligodendrocyte lineage, but the biological functions of Olig1 are not well resolved. Gain-of-function analysis suggests a role for Olig1 in oligodendrocyte progenitor development (*16*, *17*); however, Olig1$^{-/-}$ mice are viable and fertile and show only a subtle delay in oligodendrocyte maturation (*13*).

Both Olig genes continue to be expressed in mature oligodendrocytes, which suggests that they may have functions in the adult brain and spinal cord, independent of their role in development (*18*). To explore these functions, we generated polyclonal and monoclonal antibodies that specifically recognize Olig1 or Olig2 (fig. S1) and used these reagents for immunohistochemical analysis at several stages of mouse CNS development. Olig2 was localized to the nucleus at all stages examined and in all regions of the CNS (Fig. 1, A and C). In neonatal mice, Olig1 was likewise localized to the nucleus. However, Olig1 proteins were located mostly in the cytoplasm by 2 weeks after birth and were entirely cytoplasmic in the white matter of the adult mouse (Fig. 1A). In vitro studies of neonatal oligodendrocyte cultures similarly demonstrate that the subcellular localization of Olig1 becomes progressively more cytoplasmic as these cells mature into myelin basic protein positive (MBP+) oligodendrocytes (*19*). Cytoplasmic localization of Olig1 and nuclear localization of Olig2 in the adult mouse brain were confirmed by deconvolution microscopy (Fig. 1B). This pattern of subcellular localization for Olig1 and Olig2 in the adult CNS was reproduced in all areas of white matter analyzed (Fig. 1C). The differential localization of Olig1 and Olig2 is also seen in the adult human brain (Fig. 2D).

Several toxins, including cuprizone, lysolecithin, and ethidium bromide, are widely used to study the mechanisms of remyelination (*20*, *21*). As shown in Fig. 2, A to C, Olig1 proteins are initially located in the nucleus of cells in early remyelinating lesions

of the corpus callosum or of brain stem white matter after treatment with cuprizone or ethidium bromide, respectively. Similar observations were made after lysolecithin-induced injury in the spinal cord of adult mice (fig. S2). The differential localization of Olig1 in these rodent models of demyelinating disease was recapitulated in postmortem brain tissue from patients with MS (Fig. 2D). Tissue was analyzed from six cases of MS, three displaying chronic active MS lesions and three displaying chronic silent lesions (*22*). Cells containing cytosolic Olig1 were present in normal-appearing white matter. Nuclear Olig1 was present at the edges of the active lesions and of all but one of the chronic lesions. Serial sections were analyzed for the presence of mature oligodendrocytes and myelin (MBP), oligodendrocyte progenitors (NG2), and Olig1 and Olig2 protein expression. The distribution of mature and immature oligodendrocytes suggests that the cells containing nuclear Olig1 at the edge of MS lesions are likely to be undifferentiated progenitors (Fig. 2D). Colabeling localizes nuclear Olig1 to the NG2+ oligodendrocyte progenitors (Fig. 2E). Because the process of remyelination is more likely to occur in the active lesions (*23*), this distribution is consistent with a role for Olig1 nuclear translocation in the repair process in MS patients.

Functional insights into the role of Olig1 in CNS repair can be derived from analysis of Olig1$^{-/-}$ mice (*13*). The ultrastructural images of the adult CNS show that Olig1 function is not required to develop a healthy, fully myelinated brain or spinal cord (Fig. 3A). Moreover, as noted previously, Olig1$^{-/-}$ mice show no obvious abnormal behavioral phenotype. However, as seen in Fig. 3, B to D, and fig. S3, Olig1 function is required for the remyelination phase of both cuprizone (brain)– and lysolecithin (spinal cord)–induced demyelination. These data indicate a critical role for Olig1 in the repair of the adult CNS.

Why is remyelination so inefficient in the CNS of Olig1$^{-/-}$ mice? As shown in Fig. 4A, the initial event in remyelination is an appearance of oligodendrocyte progenitor cells marked by NG2, Olig1, Olig2, and Nkx2.2 (a homeodomain transcription factor) (*24*, *25*). A double immunostain with polyclonal (Olig2) and monoclonal (Olig1) antibodies shows that Olig1 and Olig2 are coexpressed in these progenitor cells (Fig. 4B). As shown in Fig. 4, C and D, and fig. S4, loss of Olig1 function has no obvious effect on the recruitment of these progenitor cells. Expression of Olig2 is maintained in these progenitors, ruling out the possibility that Olig1 is required to maintain Olig2 expression. Analysis of oligodendrocyte maturation in Olig1$^{-/-}$ animals after demyelination (Fig. 3, E and F) demonstrates that these progenitors are impaired in their ability to differentiate. Therefore, Olig1 has an essential role in oligodendrocyte differentiation and consequent remyelination in the context of white matter injury.

Another facet of the response to demyelination in both humans and rodents is the proliferation of microglia and astrocytes (reactive gliosis). As shown in fig. S5, neither reactive response is perturbed in the Olig1$^{-/-}$ mice. Thus, the role of Olig1 in the response to injury appears to be confined to oligodendrocytes and their progenitors. This separation of reactive gliosis and remyelination is in accord with several lines of evidence indicating that oligodendrocytes and astrocytes arise from separate progenitor populations (*13*, *14*).

We have shown that intracellular localization of Olig1 protein is dynamic and changes with the developmental state of oligodendrocyte lineage cells. Demyelinating injuries to the adult brain evidently create an environment that recapitulates the immature brain, allowing nuclear localization of Olig1. Because nuclear localization is important for transcription factor function, we deduce that the critical phase of Olig1 activity in oligodendrocyte differentiation occurs before it becomes localized to the cytoplasm.

With respect to the human disease, the lesions that define MS are now thought to

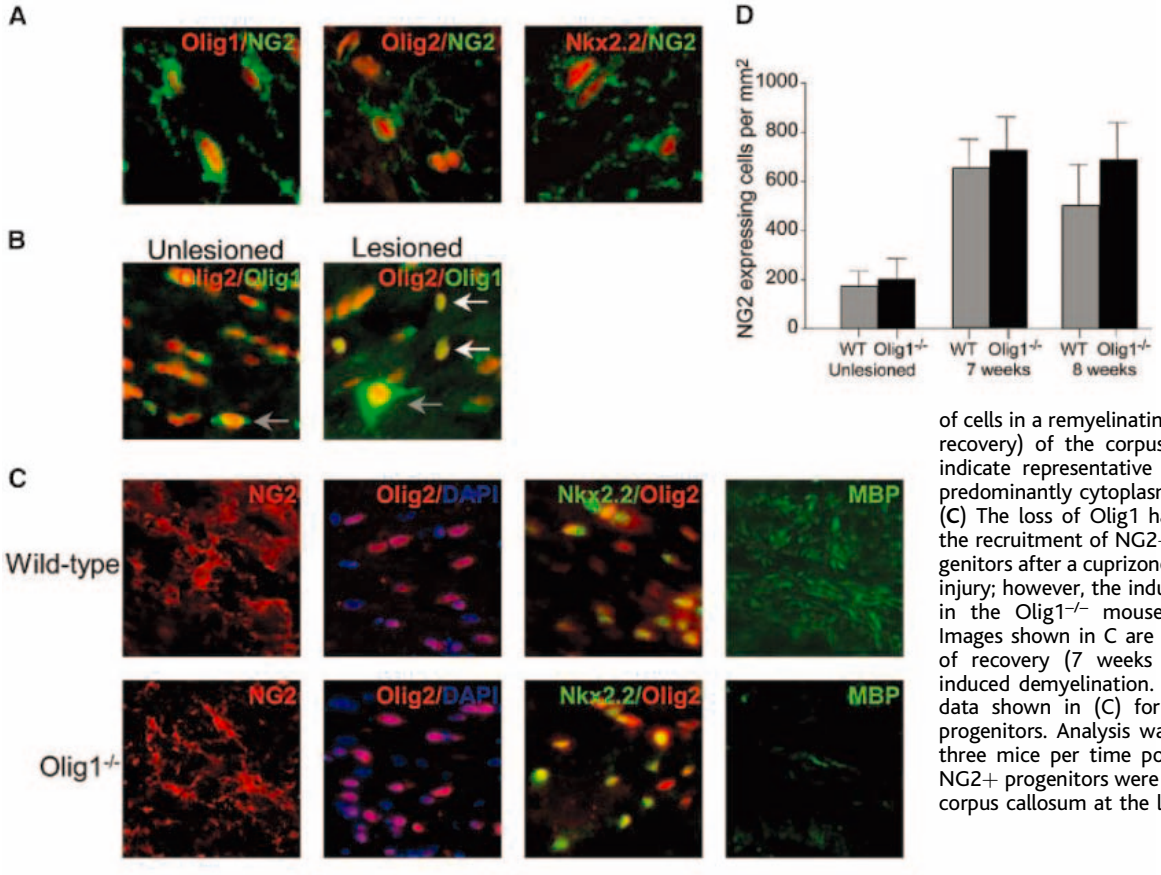

Fig. 4. Olig1 is critical for the maturation of oligodendrocyte progenitors. (**A**) After demyelination with cuprizone, cells positive for NG2, Nkx2.2, and both of the Olig genes are present in the corpus callosum. (**B**) Olig1 and Olig2 are coexpressed in normal and lesioned white matter. White arrows indicate colocalization of Olig1 and Olig2 in the nucleus of cells in a remyelinating lesion (after 1 week of recovery) of the corpus callosum; gray arrows indicate representative cells in which Olig1 is predominantly cytoplasmic and Olig2 is nuclear. (**C**) The loss of Olig1 has no obvious effect on the recruitment of NG2+/Nkx2.2+/Olig2+ progenitors after a cuprizone-induced demyelinating injury; however, the induction of MBP is delayed in the Olig1$^{-/-}$ mouse during remyelination. Images shown in C are from mice after 1 week of recovery (7 weeks total) from cuprizone-induced demyelination. (**D**) Quantitation of the data shown in (C) for recruitment of NG2+ progenitors. Analysis was performed in at least three mice per time point, and cell counts for NG2+ progenitors were restricted to the midline corpus callosum at the level of the fornix.

arise from a number of different mechanisms, mostly immunologic but not always linear or cell-mediated (*26*, *27*). Accordingly, MS may never be entirely preventable, and new therapeutic approaches must focus on the repair (remyelination) process. At the level of basic science, one important unresolved issue is why remyelination is so limited in patients with MS even though endogenous oligodendrocyte progenitors are often present in abundance (*9*, *28*). Studies shown in this paper indicate that signals regulating the subcellular localization and/or activity of Olig1 during development may play an additional and critical role in activating oligodendrocyte progenitors in the adult CNS. Our data show that requirements for Olig1 function are subtle during development, yet striking during the repair of a demyelinating lesion. This would suggest that cell-intrinsic activity of Olig1 can be compensated for during myelination but not remyelination. Further insights into the molecular mechanisms of Olig1 function during development may have practical overtones for future therapeutic interventions in MS.

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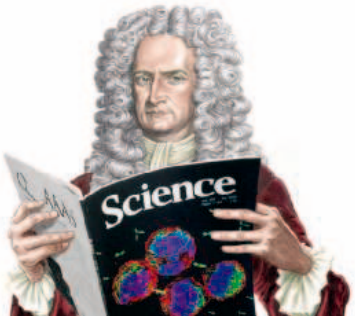

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The Department of Molecular and Microbiology (**website: http://www.gmu.edu/departments/mmb/**) of George Mason University seeks applicants for an **OPEN RANK** tenure-track position in pathogenic microbiology. The successful applicant will have postdoctoral experience, a strong commitment to teaching undergraduate and graduate students, an excellent publication record, and demonstrated ability to attract external funding. Candidates with expertise in microbial physiology, genetics, aerobiology, or functional genomics of pathogens will be considered. The successful candidate will join the biodefense effort of the University, and teach microbiology courses to undergraduate and graduate students. Applicants should apply online at **website: http://jobs.gmu.edu** attaching a letter of application, curriculum vitae, a statement of research and teaching interests, and names of three references.

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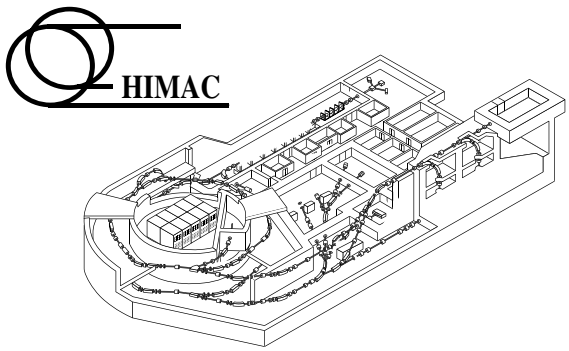
BIOLOGIST. The Department of Biology at the University of San Francisco (USF) invites applications for a tenure-track position at the **ASSISTANT PROFESSOR** level, beginning August 2005, to teach upper-division courses in the field(s) of specialty, participate in lower-division courses for majors and nonmajors, and establish an active research program. For details, please see **website: http://www.usfca.edu/hr/employment/faculty3.html.**
USF is an Affirmative Action/Equal Opportunity Employer.

ScienceCareers.org

National Institute of Radiological Sciences
Chiba, JAPAN

POSITIONS AVAILABLE

The National Institute of Radiological Sciences (NIRS), located 30 km east of Tokyo, is a unique national research institute for investigating the relationship between humans and radiation from a wide range of viewpoints. The current subjects of R&D at NIRS include the biological effects of radiation and their mechanisms, medical countermeasures against radiation injuries, and diagnosis and treatment of disease using radiation and radioisotopes. We also conduct interdisciplinary research in neuroscience, genomics and other areas. NIRS researchers with the expertise in various natural sciences conduct a wide range of R&D projects, including advanced cancer therapy using the Heavy Ion Medical Accelerator in Chiba (HIMAC).



Several positions at NIRS are available as follows: (One person for each category)

- Research Center for Charged Particle Therapy
  - Clinical PET researcher for neurotransmitter receptors and evaluation of new PET ligands (tenured position, team leader, vacancy no. 16-556 -1)
  - Clinical researcher for development of cancer imaging (fixed-term position, vacancy no. 16-556-2)
  - Researcher for development of radiopharmaceuticals (fixed-term position, vacancy no. 16-556-3)

Application Deadline: January 31, 2005 (Applications must be received by the closing date)
Date of Employment: April 1, 2005 (or later). All positions offer a competitive salary.
Fixed-term positions may lead to tenure after the end of their term.

Further details and application forms are available on our website (http://www.nirs.go.jp/ENG/nirs.htm).
For inquiries on the positions, please send an e-mail to personnel@nirs.go.jp (indicate vacancy number).

University of Tennessee System
Vice President for Research

The University of Tennessee System seeks applications and nominations for the position of Vice President for Research. The Vice President for Research reports to the President and is a member of the President's executive team. The University of Tennessee System comprises campuses in Knoxville, Memphis, Chattanooga, Martin and Tullahoma and statewide institutes of agriculture and public service. Externally sponsored research and public service projects for the System totaled $286 million in FY2004. The University intends to build its research portfolio through strategic partnerships with the Oak Ridge National Laboratory, Saint Jude Children's Research Hospital and other research agencies and organizations.

The Vice President will provide leadership to enhance and expand the portfolio of research programs of The University of Tennessee System and provide oversight for existing and newly created Research Centers of Excellence. The Vice President will be the University's lead research liaison with the Oak Ridge National Laboratory, which is managed by UT-Battelle. In this role, the Vice President will promote development of research programs that capitalize on the strengths of research teams at UT and the Oak Ridge National Laboratory. The Vice President will be a member of UT's federal relations team, will serve as the lead contact with federal research agencies and will work with the Vice President for Public and Government Relations to advocate to members of Congress. The Vice President will provide oversight and leadership for the UT Research Foundation, including expanding and leveraging the University's intellectual property. The Vice President will promote development of entrepreneurial efforts associated with UT's research programs and help build new research partnerships with the public and private sector. The Vice President will work closely with administrators from each of UT's campuses and institutes to promote collaboration and development of System-wide research priorities and initiatives.

The successful candidate for Vice President for Research must have a doctoral degree appropriate for his/her discipline; must have a record of outstanding scholarship and accomplishments in conducting competitive research in natural, physical, biomedical or mathematical sciences; must have experience in administering multi-disciplinary research programs within a major research organization, agency or university; must have experience in working with national laboratories and federal research agencies; must have a commitment to quality and integrity and must have strong skills in communications and interpersonal relations. The successful candidate must be committed and knowledgeable of affirmative action and EEO requirements. It is desirable for the candidate to have experience in a multi-campus university system, to have experience in advocating research in humanities and social sciences, and to have experience in management of joint programs between universities and national laboratories.

The Search Committee will begin reviewing completed applications on **January 18, 2004** and the search will continue until the position is filled. To be considered, submit a letter of interest, curriculum vitae and names of at least three references (include their postal or e-mail address). Those wishing to nominate candidates should submit contact information for each nominee and a brief description of each nominee's qualifications. Send applications or nominations to: **Jack Britt, Executive Vice President, 709A Andy Holt Tower, Knoxville, TN 37996-0174** or e-mail information to **jbritt@utk.edu**. Information may also be sent by FAX to **Jack Britt** at **865-974-1324**.

The University of Tennessee is an EEO/AA/Title VI/Title IX/Section 504/ADA/ADEA institution in the provision of its education and employment programs and services.

Life at Lilly

real people doing extraordinary things

Lilly is a leading, innovation-driven pharmaceutical corporation employing more than 35,000 employees worldwide. We know that the best way to find the next generation of drugs is to use the next generation technologies. And, at Lilly Systems Biology in Singapore, we're doing just that.

Here, you'll have the unprecedented opportunity to apply your knowledge of life sciences to the bioinformatics field. And you'll do so by working with tomorrow's science in a facility that redefines the leading edge.

While the rest of the industry anticipates the next generation of science, at Lilly, that's where you'll begin.

Bioinformatics Scientists

Responsibilities

- Apply state-of-the-art analysis techniques to diverse data types, including gene expression, proteomics and metabolomics
- Develop novel methods to advance data interpretation based on mathematical and statistical models of biological systems
- Work with experimental scientists on collaborative projects in drug discovery

Requirements

- Ph.D. or MS/BS with experience in applied data analysis and/or algorithm development in the Life Sciences
- Depth of knowledge in bioinformatics, biology, physics, mathematics, statistics, and/or engineering
- Computational skills in high-level programming language such as Java, C++, C, Matlab or R

Bioinformatics Developers

Responsibilities

- Collaborate with scientists/IT professionals to design, develop, and implement enterprise application systems/tools
- Communicate with users/team members to provide solutions to solve scientific problems
- Work with third party vendors/partners for joint development
- Deliver quality working software within budget and timeline
- Additional background in the life sciences/experimental medicine/clinical trails is preferred but not required

Requirements

- MS/BS Degree in Computer Science
- 5+ years of experience with enterprise application development
- Strong experience in Java, Perl, HTML, Oracle, MySQL, Unix and Linux
- Proven track record of ability to work in a new domain (e.g. Biology, etc)
- Proven track record of providing solutions for solving complex business problems
- Strong experience in architecture design and system design
- Extensive knowledge of SDLC
- Willing to do coding with demonstrated programming skills

These are exciting and challenging roles based in Singapore. Please send your resumes, in strict confidence, to: **lsb_recruitment_sg@lilly.com**.

Qualified candidates who are interested in other Bio-informatics, IT or Biology positions are also invited to visit our website.

www.lsb.lilly.com

Lilly

Answers That Matter.

Singapore Bioimaging Consortium
A*STAR

The **Singapore Bioimaging Consortium (SBIC)** is a new initiative by the Agency for Science, Technology and Research (A*STAR) that aims to use and develop state-of-the-art imaging technologies to pursue research leading to useful clinical applications. It brings together scientists and researchers from the universities, hospitals and commercial laboratories in an integrated, cutting-edge program. Four technology platforms have been identified for core research programs: optical imaging, image processing and management, small animal imaging with Magnetic Resonance (MRI and MRS) and the development of chemical/biological probes.

GROUP LEADER
LABORATORY OF METABOLIC MEDICINE
SINGAPORE BIOIMAGING CONSORTIUM

The first SBIC laboratory to be established will be the Laboratory of Metabolic Medicine (LMM). It will focus on studying mouse models of human diseases, in particular, "the metabolic syndrome" and diabetes.

You will assist in the management of a group of post-doctoral scientists and technical staff working on mouse models of human diseases. You will be attached to a laboratory at Oxford University under the guidance of Professor Sir George Radda and Professor Kieran Clarke for up to 6 months before returning to Singapore to lead the research program at LMM. You must be able to carry out independent and novel research in a highly collaborative environment. You must also help to train graduate students and post-doctoral fellows.

Requirements:
- At least 5 years' post-doctoral research experience in animal metabolism and an interest in models for human diseases
- Strong expertise in a range of analytical techniques
- Knowledge of Magnetic Resonance Imaging (MRI) and other imaging methods is an advantage
- Excellent communication and management skills
- Good track record of international publications

An attractive and competitive remuneration package is offered. This includes housing subsidy, a settling-in allowance, as well as air passage for staff and family, for the successful candidate who is either a foreigner or Singapore citizen who has worked continuously abroad for over 5 years.

Please send a full CV, with names and contact details of two academic referees, to **Dr Ngee-Chih Foo** at **Foo_Ngee_Chih@a-star.edu.sg**.

The closing date for this application is **31 December 2004**.

Enquiries on other research positions are welcome. Visit our website: www.a-star.edu.sg for more details.

Case School of Medicine
Department of Molecular Biology and Microbiology

Faculty Positions in Microbial Biology/Pathogenesis

The Department is undergoing significant expansion under our new **Chair, Dr. Jonathan Karn**. At least 6 faculty recruitments are planned over the next few years, with positions available at the **Assistant, Associate, Full Professor** levels. We encourage applications from highly qualified individuals with demonstrated experience in the fundamental aspects of Microbial Cell Biology (e.g. cell structure, growth, division, differentiation), and/or Molecular Mechanisms of Bacterial or Fungal Pathogenesis (e.g. virulence factors, biofilms, host cell interaction). Successful candidates will establish a vigorous research program, participate in teaching activities, and interact productively with basic and clinical scientists interested in microbiology and infectious diseases. We offer new laboratory space, generous start-up packages, and a highly interactive environment with exceptional intellectual, infrastructural, and administrative support. Further details are available at: **http://www.cwru.edu/med/microbio/**.

Please submit a letter of application, CV, brief statement of research goals and accomplishments, and names of 3 references to: **Piet de Boer, Ph.D., Chair; Microbial Biology/Pathogenesis Search Committee** either online at the website above, or via email to: **beo3@case.edu**.

Case Western Reserve University is an Equal Opportunity Employer.

Faculty Position
Biological Imaging
Center for Brain Science and Division of Engineering and Applied Sciences
Harvard University

Applications and nominations are invited for a tenure-track faculty position in imaging at the assistant, associate, or full professor level. We seek individuals with outstanding records of innovation in the development of new imaging or image processing techniques with potential applications to cell biology or neuroscience. We strongly encourage applications from, or information about, qualified women and minority candidates. All areas of biological imaging research are of interest, particularly: (1) innovative optical imaging techniques at the molecular, cellular, and systems level; (2) medical image processing; (3) MRI physics and novel MRI imaging techniques; and (4) other innovative imaging methods. Responsibilities include participation in Harvard's undergraduate degree programs in engineering and applied sciences, as well as in research and teaching at the graduate level. A Ph.D. is required.

Appointees will hold an academic appointment in the Division of Engineering and Applied Sciences (DEAS), which is home to Harvard's programs in applied physics, engineering, computer science, and applied mathematics. A significant enlargement of DEAS is now underway, including expansion of the faculty, new laboratory buildings, increased graduate admissions, and closer links between DEAS and Harvard's basic science departments and medical school. Further information is available at **http://www.deas.harvard.edu**.

The successful candidate will also hold an appointment in Harvard University's new Center for Brain Science, which will bring together scientists involved in research on neurons, neural networks, and behavior. The aims are to build the tools (mechanical, molecular, computational and theoretical) required to map neural circuits that underlie experimentally accessible behaviors in diverse species. Ten or more new faculty will be appointed over the next few years. The Center will foster interactions across disciplinary boundaries: faculty from several academic departments will be housed in common research space and connections will reach out across the University.

Because of the opportunity for synergistic appointments, we encourage applicants to inform us of other exceptional candidates including present or potential collaborators. For full consideration, applications should be received by **January 15, 2005**.

Please send a cover letter, curriculum vitae, copies of 1-3 publications, and the names and contact information (including email addresses) of at least 3 references to: **Imaging Search Committee , Kate Zirpolo, Division of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138.** Inquiries and applications (using pdf files please) may be directed to **imaging@deas.harvard.edu**.

Harvard is an Affirmative Action/Equal Opportunity Employer.

Assistant Professorships in Cancer and Microbial Bioinformatics
University of Georgia, Athens

The University of Georgia (UGA) seeks applications for TWO tenure track faculty positions at the Assistant Professor level. While the institution is mainly interested in receiving applications in the areas of (a) cancer bioinformatics and (b) microbial bioinformatics, applications in other areas of bioinformatics and computational biology are also welcome. Major responsibilities include establishment of an independent research program and participation in teaching of undergraduate and graduate courses. One position will be joint with the Institute of Bioinformatics and Department of Biochemistry and Molecular Biology, and the other position will be joint with the Institute of Bioinformatics and Microbiology Department. UGA will provide highly competitive start-up packages. Evaluation of applications will start on **January 17, 2005**. Applications received by February 15, 2005 are assured full consideration. Applicants must have a Ph.D. degree or other suitable terminal degree. Preference will be given to candidates who have received excellent training in both the biological and computational sciences and who possess strong computational skills to conduct *in silico* biological research in the desired areas. Applicants should electronically submit their applications, consisting of a cover letter, curriculum vitae and a short research statement, to **jy@uga.edu**. To receive full consideration, applicants should arrange to have at least three letters of reference sent to: **Faculty Search Committee Chair, c/o Ms. Joan Yantko, Institute of Bioinformatics, University of Georgia, Life Sciences Building, 120 Green Street, Athens, GA 30602-7229**.

The University of Georgia is an EEO/AA Employer.

Center for Systems Integration and Sustainability Michigan State University- Dept of Fisheries and Wildlife

ASSISTANT/ASSOCIATE PROFESSOR; ASSOCIATE DIRECTOR – ANR 1590
Fixed-Term, 9-month basis, 100% time

DUTIES: Assist the Director in conducting cutting-edge research on emerging issues related to ecological sustainability (local/national/international levels); training new generations of leaders for sustainability research, education and practice; disseminate research findings across the globe. Assist the Director in all aspects of activities, including research, education, outreach, and administration.

QUALIFICATIONS: Ph.D. in a field relevant to ecological sustainability (such as ecology, sociology, economics, human demography, remote sensing, geography, or natural resource management), a strong research record, experience in outreach, and excellent administrative skills.

SPECIALIST – ANR 1591
Fixed-Term, 12-month basis, 100% time.
AN Salary $50,000

DUTIES: Oversee and manage computer resources for the Center for System Integration and Sustainability; provide training for Center staff, students and partners regarding technology issues; mentor students; provide hardware/software/programming, database management and data analysis for research and outreach programs.

QUALIFICATIONS: Master's degree with background in computer science with previous experience in natural resources/ecology preferred. Three to five years of related and progressively more responsible or expansive work experience in providing customer support in computer hardware, software, programming, data analysis, and database management for research and outreach programs. Experience with remote sensing, geographic information systems, and spatial database is required.

APPLICATION: Interested individuals should prepare (1) letter of interest, (2) resume, (3) description of professional experiences and goals, and (4) names and contact information of three references. A copy of transcripts, GRE scores and TOEFL scores (for international applicants) should also be arranged. **Application Deadline: January 15, 2005** or until the positions are filled. Please send application materials to: **Professor Jianguo Liu, Michigan State University, Department of Fisheries and Wildlife, 13 Natural Resources Building, East Lansing, MI 48824-1222 Telephone: (517) 355-1810; FAX: (517) 432-1699; e-mail: jliu@panda.msu.edu**.

Michigan State University is an Equal Opportunity/Affirmative Action Employer. Minority and women candidates are encouraged to apply. Persons w/ disabilities have the right to request and receive reasonable accommodation.

Department of Health and Human Services National Institutes of Health National Eye Institute

Ophthalmic Genetics Branch Chief

The National Eye Institute (NEI) seeks an outstanding clinician scientist for the tenured position of Chief, Ophthalmic Genetics and Visual Function Branch (OGVFB) in the Division of Intramural Research. This recruitment is directed towards clinicians with expertise in retinal neurodegenerative disease and offers a unique opportunity for a talented individual to provide strong and stimulating leadership in an organization dedicated to uncovering new scientific knowledge, both laboratory and clinical. We welcome the full range of candidates at all levels.

The Chief, OGVFB, is expected to create a vigorous research program to elucidate mechanisms of retinal neurodegenerative disease and explore treatments. The Chief will develop broad investigational plans, independently and in collaboration with other NEI investigators and research scientists in the United States and abroad. The Chief will examine and treat patients, as well as design, implement and conduct research and clinical protocols. The OGVFB includes a molecular genetics laboratory section and provides genetic services. The Chief supervises a staff of research clinicians, basic scientists, and technicians in support of the Branch's research objectives. An opportunity exists for the Chief to recruit staff and supervise training.

The NEI provides an exceptional environment for clinical research including the infrastructure necessary for patient recruitment, a clinical protocol development group, and a Contract Research Organization that provides statistical and epidemiological expertise, data management and analysis, study monitoring, regulatory guidance, and overall operational support. The NIH Clinical Center provides additional access to exceptionally broad medical and diagnostic resources.

The position requires an ability to integrate basic, clinical and translational research, and create an intellectual synergy and an environment for state-of-the art patient care for those suffering from eye disease. The Chief will explore translational ideas in collaboration with a newly created multidisciplinary laboratory of neurodegenerative disease. This multidisciplinary laboratory is part of a complex that houses an integrated group of investigators from a diverse set of NIH Institutes, including the National Institute of Neurological Disorders and Stroke (NINDS), the National Institute of Mental Health (NIMH), the National Human Genome Research Institute (NHGRI) and the National Institute of Allergy and Infectious Diseases (NIAID). There are unparalleled opportunities for strong interdisciplinary collaborations among these independent research groups and with other investigators throughout the NIH through several on-going Trans-Institute initiatives in neuroscience, stem cell biology, gene therapy and vascular biology and angiogenesis.

At a minimum, candidates should have a Doctor of Medicine degree from a school in the U.S. or Canada approved by a recognized accrediting body in the year of the applicant's graduation, or a Doctor of Medicine or equivalent degree from a foreign medical school which provided education and medical knowledge substantially equivalent to accredited schools in the United States. Candidates should be Board-certified and have direct clinical experience in the pathophysiology of retinal neurodegenerative disease, specifically, from the perspective of understanding biological mechanisms in any of the following areas: biochemistry, cell biology, genetics or physiology.

Applicants should submit a curriculum vitae, bibliography, copies of their five most significant publications, a summary of research accomplishments and three reference letters. Applicants should also submit a written statement with their perspective on the needs and opportunities necessary to move from the basic understanding of retinal neurodegenerative diseases to clinical therapeutic interventions and improved patient care. This statement should indicate how the applicant's particular expertise and background could contribute to this transition. Applications should be sent to: **Kimberly Ruzickova, Staff Assistant, Office of the Scientific Director, National Eye Institute, Building 31, Room 6A22, 31 Center Drive, Bethesda, MD 20892; Tel: 301-451-6763, Email: Ruzickok@nei.nih.gov**. Completed applications must be received and/or postmarked by **February 28, 2005**.

DHHS, NIH and NEI are Equal Opportunity Employers.

Rensselaer

Why not Change the World?

BIOLOGY FACULTY

The Department of Biology at Rensselaer Polytechnic Institute seeks candidates in any area of basic biomedical research for tenure track faculty positions at all academic levels. We are particularly interested in candidates in the area of genomics, proteomics, and bioinformatics as part of a campus-wide initiative in computational biology and bioinformatics. Rensselaer has recently opened a 218,000 sq. ft. Center for Biotechnology and Interdisciplinary Studies with approximately 60 faculty laboratories and state of the art core facilities. Significant funding is available for startup packages. Review of applications will begin December 15, 2004, but searches will continue beyond that date. Please send a curriculum vitae, a statement of research interests up to three pages, and a minimum of three letters of reference sent to:

Robert E. Palazzo, Director,
Center for Biotechnology and
Interdisciplinary Studies
Biology 1W14 SC

Rensselaer Polytechnic Institute
110 8th Street
Troy, New York, 12180-3590

Rensselaer Polytechnic Institute is an Equal Opportunity/Affirmative Action Employer.
Women and minorities are strongly encouraged to apply.

Tenure-Track Faculty Position in Nuclear or Particle Theory at Purdue University

The Purdue University Department of Physics invites applications for a tenure track Assistant Professorship. This position is a cooperative fellowship with the RIKEN BNL Research Center at Brookhaven National Laboratory. The Assistant Professor/Fellow will divide their time equally between Purdue and Brookhaven. Candidates should have a Ph. D. in theoretical nuclear or particle physics, are expected to teach at the undergraduate and graduate levels, and should have demonstrated outstanding research ability. We are especially interested in candidates with experience in non-perturbative QCD, for example lattice QCD. Equal weight will be given to outstanding candidates in any area of particle theory. Applications to both Purdue and BNL should include a curriculum vitae, a statement of career objectives, representative publications, a statement regarding teaching philosophy, and a research plan. Electronic submission to **theory_search@physics.purdue.edu** is strongly preferred. Candidates should also arrange for at least three letters of reference to be sent to: **Faculty Search, c/o Lynn Gerrard, Department of Physics, 525 Northwestern Avenue, W. Lafayette, IN 47907-2036**. To ensure full consideration, applications should be received by **January 1, 2005**. In addition, applications should also be sent to the RIKEN BNL Research Center before January 1, 2005 (send inquiries to **rhicfellows@bnl.gov** or to **N. P. Samios, Director, RIKEN BNL Research Center, Building 510, Brookhaven National Laboratory, Upton, NY 11973**). *Purdue University is an Equal Opportunity/Equal Access/Affirmative Action Employer. Women and minorities are encouraged to apply.*

UNIVERSITY OF CALGARY

Dean of the Faculty of Kinesiology

The University of Calgary invites applications and nominations for the position of **Dean of the Faculty of Kinesiology** for a five-year renewable term commencing July 1, 2005.

The Faculty of Kinesiology

The Faculty of Kinesiology is home to a strong group of researchers, boasts some of the finest facilities in the world, and hosts one of the largest programs of its kind in Canada with more than 30 full-time faculty, 200 non-academic staff, 600 undergraduate and 65 graduate students. Programs of study are offered at the Bachelor's (nationally accredited BKin and BSc), Master's (MSc and MKin), and Doctoral levels (PhD). Kinesiology has strong ties with most faculties and significant involvement on an undergraduate, graduate, and/or research level with the faculties of Medicine, Engineering, Nursing, Education, and Business. The Faculty includes the Human Performance Laboratory, Sport Medicine Centre, Sport Technology Research Centre, Athletics, Campus Recreation, the Health and Recreation Centre, and the Olympic Oval, many of which are internationally renowned. See the Faculty of Kinesiology website at **http://www.kin.ucalgary.ca** for more information.

The Position

The Dean of the Faculty of Kinesiology will serve as a member of the senior leadership team of the University and is expected to have:

- an accomplished research record with a PhD in Kinesiology (or in a related discipline);
- a vision that recognizes and values the various disciplinary commitments of the Faculty;
- proven leadership abilities and administrative experience in an academic or similar setting;
- successful fundraising experience and demonstrated financial acumen; and
- the ability to foster relationships with external stakeholders (research communities, alumni, and local community groups).

The ideal candidate will seize the opportunities of an energetic and dynamic Faculty to:

- enhance the national and international research and educational reputation of the Faculty;
- advocate the place of Kinesiology in the development of scientific knowledge and the advancement of the human condition through the natural, medical, and social sciences;
- lead this ambitious Faculty to an even higher level of worldwide recognition;
- secure additional sources of funding through research funding bodies, including Alberta Heritage Foundation for Medical Research, Alberta Ingenuity Fund, Alberta's Informatics Circle of Research Excellence (iCORE), and Canada Foundation for Innovation; and
- strengthen the Faculty's position relative to other health and wellness faculties and units at the University.

University of Calgary

The University of Calgary is a comprehensive research-intensive university with almost 30,000 undergraduate and graduate students. Situated close to the Canadian Rockies, in a rapidly growing city characterized by diversity, volunteerism and entrepreneurial vitality, the University helps the province foster discovery and innovation and develop a highly skilled workforce for the knowledge economy.

Applications, complete with an expression of interest, curriculum vitae, and the names of three referees, who have been asked to write in confidence to the committee, should be forwarded to: **Dr. Ronald Bond,** Provost and Vice President (Academic), University of Calgary, 2500 University Drive N.W., Calgary, Alberta, Canada, T2N 1N4, Phone: (403) 220-5464, Fax: (403) 289-6800, Website: http://www.ucalgary.ca

Consideration of applications will begin in mid-February 2005. The competition will remain open until a suitable candidate is found.

All qualified candidates are encouraged to apply; however, Canadians and permanent residents will be given priority. The University of Calgary respects, appreciates and encourages diversity.

To see all University of Calgary academic positions, please visit www.ucalgary.ca/hr/career

www.ucalgary.ca

University of Zurich

The University of Zurich Faculty of Medicine invites applications for the post of

Professorship in Pharmacology/ Chronobiology and Sleep-Research

at the Institute of Pharmacology and Toxicology tenable from September 1, 2006.

The appointee will be expected to have an outstanding record of research within the field of chronobiology, preferentially related to neurobiology and neuropharmacology. Present and future research activities should have the potential for interdisciplinarity including collaborations with clinical disciplines.

The successful candidate is expected to participate in under- and postgraduate teaching in medicine, biology and pharmacy. Thus, appropriate teaching skills and experience are essential.

It is planned to fill the position at a tenure-track assistant-professor level. However, depending on qualifications and previous experience a tenured full professorship is possible.

Written applications *(in duplicate)* for this post should be addressed to the University of Zurich Faculty of Medicine, Berufungskoordination, Zuerichbergstrasse 14, CH-8091 Zuerich no later than January 31st 2005. For informal enquiries please contact the President of the Appointments Board, Prof. Dr. Heini Murer, Physiologisches Institut, Winterthurerstr. 190, CH-8057 Zuerich (phone +41-1-635 50 30).

The application must include the particulars listed in the "Instructions for Submitting Applications". The guidelines are available from the Dean's Office of the Faculty of Medicine (Fax +41-1-634 10 79) or at **http://www.med.unizh.ch/dekanat/richtform.html**

IB-CAS

Principal Investigators (4) Institute of Botany, Chinese Academy of Sciences, Beijing

The Center for Plant Signal Transduction and Metabolomics (C-STM) is a newly established research center in the Institute of Botany, Chinese Academy of Sciences, aiming to understand fundamental questions on plant development and defense. We are seeking 4 outstanding scientists to fill the positions of Principal Investigators (at the full-professor level), to set up their own independent groups. Metabolomics is a high-priority area. Candidates must have a Ph.D degree, a Chinese citizenship, a demonstrated record of excellence in research, and acceptable management skills. The positions open immediately, and will be closed when the positions are filled.

The starting package includes 250 M^2 lab and office space, a supporting lab manager, an annual salary of 100,000-150,000 RMB plus housing subsidies, and 1.5-2 million RMB start-up funds. All PIs are expected to actively attract external funding. The institute is located in a scenic botanic garden at the Fragrant Hill of Beijing, with excellent transport routes. For more information, visit:

http://www.ibcas.ac.cn/ib.swf

No recommendation is needed until the second phase of the selection. Applicants should submit a curriculum vitae and a future research plan to: Dr. Lin Zhanbing, Personnel Department, Institute of Botany, Chinese Academy of Sciences, Fragrant Hill, Nanxincun No. 20. Beijing 100093, Email: **linzb@ibcas.ac.cn**

Max Planck Institute for Demographic Research

Directors: Prof. James W. Vaupel - Prof. Jan M. Hoem

The Max Planck Institute for Demographic Research is seeking to expand further its activities in the field of

Evolutionary Biodemography

and is recruiting to vacancies at the

PhD, Post-Doc, and Research Scientist levels

The successful candidates will complement an existing research team of 14 staff, working alongside a total of some 80 employees from diverse backgrounds engaged in a range of issues in demography. The team aims to gain a fundamental understanding of demographic processes, and is particularly interested in how theses are shaped by evolution. We seek to advance our knowledge of life histories using a variety of field and laboratory based studies, theoretical modeling, and analysis of existing databases. As well as studies on birds, mammals, plants and a range of invertebrate organisms, the institute is particularly well placed to support the quantitative aspects of work in this field. There are ongoing projects on age-specific schedules of mortality, reproduction and growth, on the evolution of senescence, on reproductive effort, parental investment and intergenerational transfers, and on the costs of reproduction and the delayed effects of stress.

We are seeking able scientists from all levels with strong academic track records in life history biology, ecology, demography, mathematics or statistics. Applications should be addressed to Executive Director, Prof. James W. Vaupel and should include a CV with a statement of academic interests and relevant experience, a list of publications and the contact details of 3 referees. All material should be e-mailed to: **Evodemo.positions@demogr.mpg.de.** See www.demogr.mpg.de for information.

The Max Planck Society wishes to increase the share of women in areas where they are underrepresented, and strongly encourages women to apply.

The Max Planck Society is committed to employing more handicapped individuals and especially encourages them to apply.

Evo-Demo Positions, Attn. Prof. James W. Vaupel
Max Planck Institute for Demographic Research
Konrad-Zuse-Strasse 1, D-18057 Rostock, Germany
E-mail: Evodemo.positions@demogr.mpg.de

MAX PLANCK SOCIETY

University of Oxford

in association with New College

University Lecturership in Zoology (Evolutionary Biology)

Applications are invited for the above post, tenable from 1st April 2005 or as soon as possible thereafter. The successful candidate will be offered a Tutorial Fellowship at New College. The combined college and university salary will be according to age on a scale up to £45,707 p.a. (as at 1st August 2004).

The University and the College are seeking candidates with a proven record of scholarship and research in evolutionary biology and a track record of attracting research funding. The appointee will be required to engage in research which will contribute to the department's research reputation; to teach, supervise and examine undergraduate and graduate students; and to contribute to administration in college and department.

Further particulars are available from http://www.zoo.ox.ac.uk or from Professor P Harvey, FRS, Department of Zoology, South Parks Road, Oxford OX1 3BS, e-mail: paul.harvey@zoo.ox.ac.uk Applications (electronic copies are not acceptable) including a curriculum vitae, a list of principal publications and the names and contact details of three referees (eight copies except from candidates overseas who need send only one) should be sent, for receipt not later than 4th January 2005. There is no application form, and separate application is not required for the college post.

The University is an Equal Opportunities Employer.

The University of Dublin

TRINITY COLLEGE

Department of Surgery

Lecturership in Surgery

Applications are invited for the post of Lecturer in Surgery (non-clinical) in the Academic Dept of Surgery TCD based at the Adelaide and Meath Hospital, incorporating the National Children's Hospital. This is a new post and is seen as pivotal to the development of a molecular programme on the AMNCH campus.

The Department which has a major clinical and research interest in gastrointestinal diseases seeks the appointment of a senior research scientist for this post to spearhead molecular biological investigations in a wide range of tumours including pancreas, colon, breast, cervix etc. The appointee will be expected to have a substantial publication record in peer review journals and have a strong grant income generation record. Laboratory management and grant contract negotiation experience is also required.

The appointee will work in collaboration with approximately 25 other scientists in the Departments of Medicine and Histopathology, as part of collaborative research efforts currently under way within the University. The range of facilities available to the appointee are as follows: PCR, in-situ hybridisation, cloning, cell culture, in-cell PCR, CGH array analysis, mFISH, automated karyotyping, cDNA analysis including Affymetrix and Celera/ Applied Biosystems platforms.

Appointment will be made on the Specialist Registrar scale.

Informal enquiries should be made to: **Professor Kevin Conlon, Professor of Surgery, Adelaide and Meath Hospital, incorporating the National Children's Hospital. Tel: (+353 1) 608 3719**

Closing date not later than: **12 noon on Wednesday, 5th January, 2005.**

Full application details are available on the website at **www.tcd.ie/staff_office**

Applicants should submit a full curriculum vitae, to include the names of three referees, to:

Recruitment Executive, Staff Office,
Trinity College, Dublin 2.
Tel: (+353 1) 608 1962;
Email: raffertr@tcd.ie

Trinity College is an equal opportunities employer.

National University of Ireland, Galway
Ollscoil na hÉireann, Gaillimh

National Centre for Biomedical Engineering Science

CHAIR OF MOLECULAR MEDICINE

As part of its strategic plan, National University of Ireland, Galway is committed to establishing itself as a leading research University in selected areas predicted to have significant global impact in the coming years.

The University has recognised strengths in biomedical engineering science and regenerative medicine, and has played a leading role in the growth of the healthcare sector of the economy. Established in 1999, the National Centre for Biomedical Engineering Science (NCBES) is an interdisciplinary biomedical research Institute drawing together researchers from medicine, science and engineering under the leadership of Professor Terry Smith. The Centre is accommodated in a new flagship building in the centre of campus. In 2003 the Regenerative Medicine Institute (REMEDI) at the NCBES was established under the leadership of Professor Tim O'Brien.

Building on this €54m investment - and supported by the Higher Education Authority and Science Foundation Ireland - the University continues to promote the development of new capabilities, which will give a lead to the process of evolution of research, nationally and internationally.

NUI, Galway has recently received private funding for a new position, up to and including a Professorial-level appointment. A request for expressions of interest in this post is now extended to outstanding individual researchers, with world-class research programmes in Molecular Medicine. Priority will be given to individuals who can demonstrate research leadership and vision, and to complement and provide synergies with the existing priority research clusters within the NCBES. Preference will be given to candidates with research interests in the following areas:

- Biomaterials and tissue engineering
- Functional genomics or proteomics
- Cardiovascular science
- Cancer

In the first instance, candidates should request an information pack from ***Ms Orla Baxter at the Centre (Tel: +353-91 493729)*** or visit the website at ***www.nuigalway.ie/ncbes***

Expressions of interest, including a curriculum vitae and a 5 page summary of research plans, should be submitted to ***Professor Terry Smith, Director, NCBES,*** by ***14th February 2005*** at the latest. The University will enter into discussions with candidates, which may lead to appointments in this area.

NCBES National Centre for Biomedical Engineering Science National University of Ireland, Galway

NDP

DANA-FARBER CANCER INSTITUTE

Dedicated to Discovery...Committed to Care.

CHEMICAL BIOLOGY FACULTY

The Department of Cancer Biology at Dana-Farber Cancer Institute and the Department of Biological Chemistry and Molecular Pharmacology at Harvard Medical School seeks applicants for a tenure-track faculty position. We will consider outstanding applicants interested in any area of chemical biology relevant to cancer, but we are most interested in candidates using approaches complementary to those of the current faculty at DFCI-HMS. Additionally, candidates who are developing or applying novel molecular approaches for biological imaging, for understanding signaling and other pathways, or for constructing novel materials to probe cellular and molecular function are of special interest. The successful applicant will be expected to develop a strong, independently funded research program and to participate in the teaching mission of the Institute and Harvard Medical School. Candidates must hold a Ph.D. and/or M.D. degree and have a strong record of research accomplishments. Note that exceptional candidates without extensive postdoctoral experience may be considered.

Candidates should submit a curriculum vitae including a full list of publications, a brief statement of previous contributions and future research plans as well as the names and contact information of four references to:

Melitta King, Chemical Biology Search Committee
Room C-213, Harvard Medical School, Boston, MA 02115.
Applications must be received by December 30, 2004.

The Dana-Farber Cancer Institute is an Equal Opportunity Employer.

HARVARD MEDICAL SCHOOL

SHARE THE VISION... FIND THE CURE

MILLERSVILLE UNIVERSITY — PLANT PHYSIOLOGIST
BIOLOGY DEPARTMENT

Assistant Professor, full-time, tenure-track beginning in August 2005 (Fall term). The ideal candidate can a) teach undergraduate courses in introductory botany and introductory biology, b) teach plant physiology and an upper-level course in an area of specialization, c) co-teach a course in plant biochemistry, and d) supervise undergraduate research.

Required: Ph.D. in botany/biological sciences with broad (from whole plant to molecular level) training and experience in plant physiology, teaching experience at the undergraduate level and a strong commitment to liberal arts education, a good general knowledge of biology, excellent communication skills, publications of original research that makes use of current molecular techniques to investigate the physiological basis of plant function, and a successful interview and teaching demonstration.

Preferred: Candidates with teaching and/or research experience beyond the doctorate, with a record of scholarly presentations at professional meetings, and with training and experience that would permit them to participate in a cellular and molecular techniques course. It is desirable that the candidate has interests that complement existing programs in the department with 19 full-time faculty positions and over 500 undergraduate majors.

The new and renovated science complex contains modern facilities including walk-in plant growth chambers, greenhouses, a scanning electron microscope, a state-of-the-art herbarium, plus computer and audiovisual equipment.

Full consideration will be given to applications received by **January 14, 2005**. No electronic submissions accepted. To apply, please submit hard copies (paper) of the following items: 1) statement of research and teaching interests, including documentation of previous teaching experience/ performance, 2) current curriculum vitae, 3) copies of transcripts, 4) recent published papers and manuscripts in press, and 5) three current letters of reference (at least one of which addresses teaching skill), sent by the referee, to: **Dr. David Dobbins, Chair, Plant Physiologist Search Committee/SCI1217, Department of Biology, Millersville University, P.O. Box 1002, Millersville, PA 17551-0302.**

An EO/AA Institution • www.millersville.edu

COLUMBUS STATE UNIVERSITY
Two Faculty Positions: Director and Assistant Professor Environmental Sciences Program

Columbus State University invites applications for **Program Director** of Environmental Science and an **additional full-time faculty position** to support a masters program in the College of Science. Successful candidates will hold tenure-track appointments in biology, chemistry, or geology, which provide most of the teaching faculty for the program. A successful candidate for Program Director will be expected to administer the program, direct student recruitment, maintain active, funded research, and teach about 6 hours per semester. Candidates for the additional faculty position will be expected to maintain a suitable level of research and funded activity and teach about 9 hours per semester. Both positions are tenure-track.

Candidates should be able to coordinate with existing department strengths and should possess expertise in a discipline that can be successfully funded in this region. Historic success has come to projects related to water resources and species of concern. We will consider expertise in aquatic chemistry, aquatic ecology, hydrology, landscape ecology and physical geology. Candidates presenting other combinations of expertise will be considered.

Columbus State University is a unit of the University System of Georgia, located in a suburban setting approximately 100 miles southwest of Atlanta. Current enrollment is 7200 students with 50 undergraduate and 35 graduate degree programs. The Environmental Science Program is a unit of the College of Science, the largest college within the university. For more detailed information regarding the program and the institution, please visit **http://cos.colstate.edu/**.

Review of applications will begin January 15, 2005, and continue until the position is filled. Starting date is August 15, 2005. Qualified applicants should send complete curriculum vitae, statement of research and teaching interests, and three letters of reference. Applicants are also expected to address research interests and potential sources of future funding in their application letter. Incomplete application packets will not be considered. Send applications and supporting materials to: **George E. Stanton, Dean of Science, Columbus State University, Columbus, GA 31907**.

Columbus State University is an Affirmative Action/Equal Opportunity Employer. Qualified women, minorities, and individuals with disabilities are encouraged to apply.

CHAIR, DEPARTMENT OF LABORATORY MEDICINE
UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

The School of Medicine of the University of California at San Francisco is seeking applications for the position of Professor and Chair of the Department of Laboratory Medicine. We seek an individual of vision who is committed to the Department's goal of becoming a leading force in the development and application of new technologies for the diagnosis and management of human disease.

Applicants should have an MD, PhD or MD and PhD degree(s) and have a nationally recognized research program in basic or translational research. The successful applicant will play a leading role in building cross-cutting translational research programs at UCSF; will become a member of the Biomedical Sciences Graduate Program at UCSF; and will participate actively in the mentoring of fellows and residents in Laboratory Medicine, Pathology and other translational research disciplines.

Applicants should submit a CV and brief statement of research interests and plans by **15 January 2005**. Submit a hard copy and an electronic copy to:

Don Ganem, MD
Laboratory Medicine Search Committee Director
c/o Michael Armanini
G.W. Hooper Foundation, Box 0552
University of California
513 Parnassus Ave
San Francisco, CA 94143
e-copies to : Armanin@itsa.ucsf.edu

UCSF is an Affirmative Action/Equal Opportunity Employer. The University undertakes affirmative action to assure equal employment opportunity for underrepresented minorities and women, for persons with disabilities, and for Vietnam-era veterans and special disabled veterans.

ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

Faculty Positions in Bioinformatics

at Ecole Polytechnique Fédérale de Lausanne (EPFL)

The School of Computer and Communication Sciences and the School of Life Sciences at EPFL invite applications for faculty positions in bioinformatics at the **tenure track assistant professor and tenured associate and full professor levels.**

Successful candidates will develop an independent and creative research program, participate in both undergraduate and graduate teaching, and supervise PhD students. The language of instruction in the graduate school is English.

Candidates from all areas of bioinformatics will be considered, but preference will be given to candidates with interests in **drug design, genomics, phylogenetics and protein modeling.**

Salaries are internationally competitive. Positions come with substantial institutional resources, at all ranks.

To apply, please follow the application procedure at http://bioinformatic-recruiting.epfl.ch. The following documents are requested in PDF format: curriculum vitae, including publication list, brief statements of research and teaching interests, names and addresses (including e-mail) of 3 references for junior positions, and 6 for senior positions.

Screening will start on **February 15, 2005.** Further questions can be addressed to:

Professor Willy Zwaenepoel
Dean
School of Computer and Communication Sciences
EPFL
CH-1015 Lausanne, Switzerland
recruiting.ic@epfl.ch

For more information on EPFL, see http://www.epfl.ch

EPFL is an equal opportunity employer.

南開大學 Nankai University

Job Title: Professor (Specially-appointed Professorship)

Employer: Nankai University **Location: Tianjin, China**

Nankai University invites applications for professors (Specially-appointed Professorships) with a strong preference for candidates at the internationally renowned professors level. Specially-appointed Professors system aims at attracting distinguished people in various fields to come to Nankai.

Founded in 1919, Nankai University is located in Tianjin. It is a multi-disciplinary, comprehensive key university under the direct administration of the Ministry of Education. At present Nankai has 1527 teachers. And 12 young teachers have been selected by the Ministry of Education (MOE) as Specially Appointed Professors. For more information, please visit our website: **http://www.nankai.edu.cn**

Candidates for the position must be well-informed in relevant fields of research and well-known for their remarkable achievements in international circles, with extensive publications (as first author or correspondence author) in the world's first-class academic journals. And have working experience on the correspondent position.

Applicants for the positions must in principle have a doctorate degree. Applicants(of natural science) should be younger than 45; Applicants (of liberal art) should be younger than 50. Those who have most excellent achievement are not limited to the conditions mentioned above (but have to be confirmed and approved by the university). Specially-appointed Professors are faculty members of Nankai University. They promise to work in Nankai University totally or at least 9 months per year.

Salary is commensurate with qualifications and based on Nankai University's pay scale. Candidates should send curriculum vitae, list of publications, reprints of up to three representative papers, and a summary of research plans to: **Hongmei GAO, Personnel Office, Nankai University, Tianjin, China. Mail Code: 300071. (email: gaohm@nankai.edu.cn; phone: 0086-22-23508595; fax: 0086-22-23508595).** Candidates should also arrange to have three letters of reference sent under separate cover.

The deadline for applications is March 10, 2005, but until position is filled, all applications received will be assured full consideration. Nankai University is an equal opportunity, affirmative action educator and employer.

Job Title: Faculty

TEDA School of Biological Sciences and Biotechnology

TEDA School of Biological Sciences and Biotechnology, Nankai University, was established in February, 2004. The school is located in the Tianjin Economic and Technological Development Area (TEDA). Current researches are focused on genomics and functional genomics studies of microorganisms, with special interests in the area of bacterial surface O antigens and functional study of a thermophilic *Bacillus sp.* through whole genome sequencing, bioinformatics, proteomics and conventional molecular biology techniques.

Exciting career opportunities are available for talented professionals to join our school. The successful applicants should have a Ph.D. and /or M.D. degree with extensive leadership and scientific experience in microbiology, molecular biology or bioinformatics. The potential fields of expertise include **genomics, functional genomics, proteomics and related areas.** Excellent leadership, interpersonal communication, team-building and problem-solving skills are expected. We are also interested in candidates whose research can interact productively with the existing programs.

Lab space will be provided in a new, state-of-the-art facility and a professional working environment. Salary will be commensurate with qualifications and experience.

Applicants should submit a cover letter describing research interest and research plans, a *Curriculum Vitae*, a list of publications, copies of five representative publications, and names and contact information of at least three persons from whom references will be obtained to:

Lei Wang, Ph.D. Professor and Dean,
TEDA School of Biological Sciences and Biotechnology,
Nankai University, 23# Hongda Street,
TEDA, Tianjin 300457, P. R. China

Tel: +86-22-66229592
Fax: +86-22-66229596
Email: wanglei@nankai.edu.cn

Positions will remain open until filled.

MILLERSVILLE UNIVERSITY

ICHTHYOLOGIST
BIOLOGY DEPARTMENT

Assistant Professor, full-time, tenure-track beginning August 2005 (Fall Term). The selected candidate will: a) teach ichthyology and an advanced course in the candidate's specialty, b) teach undergraduate courses in introductory biology, and c) involve undergraduates in research with a field orientation and quantitative approach.

The Biology Department presently consists of 19 full-time faculty and over 500 undergraduate majors, and is housed in a new and renovated science complex. Millersville University is a founding member of the Marine Science Consortium, a 15-member marine laboratory with teaching and research facilities located in Wallops Island, VA; and is home to the MU Center for Environmental Science, an organization dedicated to research, education, and the long-term management and protection of natural resources in the lower Susquehanna region.

Required: Candidates must have completed a Ph.D. in a biological science, sufficient background to teach a course in ichthyology and introductory biology courses, teaching experience at the undergraduate level, excellent communication skills, demonstrated skills in field identification of the fish fauna in the Mid-Atlantic region, and a successful interview and teaching demonstration.

Preferred: Preference will be given to candidates with teaching and/or research experience beyond the doctorate; a record of scholarly presentations at professional meetings and publications in refereed journals; experience in writing grant proposals; and sufficient background to teach evolution, biometry or developmental biology.

Full consideration will be given to applications received by **January 3, 2005**. No electronic submissions accepted. To apply, please submit a letter of application and the following: 1) statement of teaching and research interests/goals including evidence of commitment to teaching, 2) current curriculum vitae, 3) copies of transcripts, 4) recent published reprints, and 5) three current letters of reference sent by the referee (at least one letter should addresses potential teaching skills and ability to supervise undergraduate research) to: **Dr. John R. Wallace, Chair, Search Committee/SCI1217, Biology Department, Millersville University, P.O. Box 1002, Millersville, PA 17551-0302.**

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esf — State University of New York College of Environmental Science & Forestry

ASSISTANT PROFESSOR FOREST PATHOLOGY

The Faculty of Environmental & Forest Biology of the State University of New York, College of Environmental Science & Forestry, in Syracuse, NY invites applications for an Assistant Professor position in Forest Pathology in support of its program in forest health.

A Ph.D in plant pathology or related discipline is required. The successful candidate is expected to develop a strong, internationally recognized, and externally funded research program in one or more of the following areas: use of landscape scale survey and quantitative approaches to the study of tree disease, insect impacts, and emergent epidemics; forest health monitoring; wood decay and wood microbiology; tree physiology and genetics as related to defense against pathogens and insects; or tree disease epidemiology. Significant contributions to the undergraduate and graduate education and service missions of the college are expected.

Teaching duties will include an undergraduate lecture and laboratory course in forest and shade tree pathology or comparable offering, a graduate seminar, and upper division/graduate level courses in special areas of interest (e.g. forest health monitoring, tree defenses against pathogens and insects, tree disease epidemiology). Effective collaboration with colleagues in forest pathology, mycology, entomology, ecology, microbiology, conservation biology, wood products engineering, and silviculture is expected. We seek excellence and enthusiasm in teaching, research, and outreach.

Applicants should send statements of research interests and teaching philosophy, CV, coursework transcripts, and arrange to have three letters of reference sent by FEBRUARY 15, 2005 directly to: **Office of Human Resources, ATTN: Forest Pathology Faculty Position, SUNY-ESF, 217 Bray Hall, 1 Forestry Drive, Syracuse, NY 13210-2778, USA.**

SUNY-ESF is an Affirmative Action/Equal Opportunity employer.

Visit ESF on the web at www.esf.edu

SUNY-ESF is an Equal Opportunity/Affirmative Action employer.

Proteomics Core Manager

The UC Davis Genome Center integrates experimental and computational approaches to address key biological problems in genomics. The Center is being housed in a new research building with state-of-the-art computational and laboratory facilities collocated with Biomedical Engineering and School of Medicine. The Center will be made up of 17 faculty and 5 technology service cores. The faculty and service cores will contribute to an internationally recognized program in genomics research at Davis, building on and enhancing the unique strengths and unmatched breadth of the life sciences on the UC Davis campus, **http://genomics.ucdavis.edu**.

The **Proteomics Core** will provide a broad range of proteomics services to diverse research groups on campus with particular emphasis on the analysis of macromolecular complexes and the post-translational modification of their constituents. This service core will operate on a recharge basis to enable research groups across campus with state-of-the-art proteomics capabilities.

The Genome Center invites applications for a **Proteomics Core Manager** who will be involved in the implementation of a campus-wide proteomics initiative. This position will have responsibility for the daily operation of the Proteomics Core facility, which includes but is not limited to the recruiting, training and management of the staff and budgetary aspects of the core. This position will also be responsible for overseeing sample preparation and scheduling, operation and maintenance of equipment, evaluation and implementation of new technologies, training of researchers when appropriate, and preparation of grant proposals to maintain the core with state-of-the-art equipment and techniques. The individual is expected to actively collaborate in the research efforts of campus faculty.

This position requires a Ph.D. or equivalent in Chemistry, Biochemistry, or Molecular Biology with at least two years postdoctoral experience. The individual should have a demonstrated knowledge and experience in mass spectrometry. The incumbent should possess broad chemical, biochemical, and technical knowledge sufficient to supervise the technical staff, troubleshoot problems, refine technologies, and advise faculty and their research groups. The position will be open until filled; for full consideration applications should be complete by **January 15, 2005**. Applicants should apply on line at **http://genomecenter.ucdavis.edu** and arrange for at least three letters of recommendation to be submitted on line.

The University of California is an Affirmative Action/Equal Opportunity Employer.

BERNSTEIN CENTER FOR COMPUTATIONAL NEUROSCIENCE BERLIN

The newly founded Bernstein Center (www.bccn-berlin.de) invites applications for several

positions for graduate students (BAT IIa/2) and postdoctoral fellows (BAT IIa)

to join the Center's research projects. The Bernstein Center Berlin was established in September 2004 by the German Ministry for Education and Science to promote interdisciplinary research and education in Computational Neuroscience through collaborative research and teaching activities between theorists, experimentalists and clinical researchers. The Center currently consists of twenty research groups from seven universities and research institutions within Berlin - Humboldt-Universität zu Berlin, Freie Universität Berlin, Technische Universität Berlin, Charité, Fraunhofer FIRST, the Max-Delbrück-Center, and the Wissenschaftskolleg zu Berlin.

Our activities range from investigations of single synapses and networks to human neuroscience, from electrophysiology and imaging to psychophysics, from mathematical theory and computational modelling to in-vivo experiments and clinical studies. The Center provides an intellectual environment for solving problems that transcend traditional disciplinary boundaries, combining modern biomedical, physical, and computational sciences, and excellent training opportunities for young researchers.

The successful candidates will be directly associated with one research group and its home institution as well as with the joint Center. Positions are initially for two years, but most positions can be extended for a longer period.

Applications should include a curriculum vitae, copies of the relevant university certificates (Master/PhD), a list of publications, and a statement of research interests. The application material and two letters of reference should be sent to the contact address specifically given for each project at http://www.bccn-berlin.de/positions.

The Center's institutions are equal opportunity employers, committed to the advancement of individuals without regard to race, colour, religion, sex, age, national origin, ethnicity, disability or any other protected status.

OncoRay is seeking for you!

Dresden University of Technology
Medical Faculty Carl Gustav Carus
Center for Innovation Competence in Radiation Oncology - OncoRay

OncoRay is a newly established interdisciplinary research center and one of the six awardees of "Center for Innovation Competence" of the 2004 "Create Excellence - Foster Talent" program of the German Federal Ministry of Education and Research. OncoRay's vision is to improve the treatment of cancer by means of biologically individualised, technically optimised radiotherapy. Focus and strategy are aimed at preclinical and clinical translational research. Three complementary and closely interacting groups of scientists will be established: "Biological and Molecular Targeting", "Biological and Molecular Imaging", "Biologically Adapted Treatment Planning and Delivery". OncoRay is jointly operated by the Dresden University of Technology and the Research Center Rossendorf and is established at the Medical Faculty. Working language of OncoRay is English.

OncoRay is seeking young outstanding scientists for

Research group "Biological and Molecular Targeting"

Molecular Biologist - Experiences in genomics, proteomics, and phospho-proteomics, manufacture of transgenic and knockout animals
Pathologist - Experiences in tumor pathology, molecular pathology, animal models, experimental radiation biology; the candidate should have board certification in nuclear medicine
Computer Scientist - Experiences in bioinformatics, biostatistics, biological-mathematical modelling
PhD-Student (Biology, Physics) - Experiences in in-vitro studies in biological and molecular targeting, cell culturing, and molecular biology
Technical Assistant - Experiences in cell culturing, and molecular biology, lab structuring
or - Experiences in animal studies, histological staining, microscopy incl. confocal laserscan microscopy

Research group "Biological and Molecular Imaging"

Nuclear Medicine Specialist - Experiences in PET, as possible PET-CT, strong interest in clinical and animal research; candidate should have board certification in nuclear medicine
Scientist (e.g. Biologist) - Experiences in animal studies on molecular and biological imaging of tumors during radiotherapy with PET, CT, MRT
(Medical radiation) Physicist - Experiences in sequential programming, coil technology, imaging processing; Scanner: 1.5 T clinical MR, 7 T experimental MR
PhD-Student - Experiences in animal studies on molecular and biological imaging of tumors during radiotherapy with PET, CT, MRT
Technical Assistant - Duty at PET-CT, high skills in organization and communication
or - Experiences in animal experiments at MRT (1.5 T and 7 T), PET, CT (molecular imaging)

The positions are funded initially for 5 years (subject to the availability of resources and administrative approval).

The Dresden University of Technology is an equal opportunity employer. Women are especially invited to apply. Preference will be given to disabled applicants with the same qualifications.

Please send your application and CV until **January 30, 2005** to: **Stefan Pieck, Coordinator OncoRay, Department of Radiation Oncology, Medical Faculty Carl Gustav Carus, Dresden University of Technology, Fetscherstr. 74, 01307 Dresden, Germany.** For further information: **ONCORAY@mailbox.tu-dresden.de** or **www.oncoray.de**

National Institute of Child Health and Human Development
National Institutes of Health
Department of Health and Human Services

Chief, Reproductive Biology and Medicine Branch

The Division of Intramural Research, NICHD is seeking an individual to direct the Reproductive Biology and Medicine Branch. The mission of the Branch is to conduct basic and clinical research in reproductive biology with a clinical emphasis on reproductive endocrinology and basic research in processes required for female and male reproductive success. The research agenda for the basic component is broad and encompasses implantation biology, ovarian and endometrial physiology and endocrinology, and stem cell biology.

Prerequisites are an M.D. or Ph.D. degree, or both, with expertise and an established track record of accomplishments required to provide the desired leadership. The Chief will report directly to the Scientific Director, Dr. Owen Rennert and will develop, organize, implement and direct a technically complex and rigorous translational research program. The Branch Chief oversees the programs of Senior Investigators and develops his/her own program on subjects of relevance to the Branch and also provides intellectual leadership and mentorship to tenured and tenure track scientists and trainees. The Branch has a training program in Reproductive Endocrinology approved by the American Board of Obstetrics and Gynecology and an Adult Endocrinology Fellowship Program.

The successful candidate will be offered a competitive salary and benefits package commensurate with experience and qualifications. Appointees must be US citizens, resident aliens or nonresident aliens with a valid employment visa.

Applications must be received by **February 15, 2005**. Please send a letter describing your scientific background and interests, along with curriculum vitae and arrange for three letters of reference to be sent to: **Roberto Romero, M.D., Chief, Perinatology Research Branch, Chair of the Search Committee, C/O AMB, DIR, NICHD, NIH, 6705 Rockledge Drive, Suite 8000, Bethesda, MD 20892-7970**. Or e-mail to **warfiela@mail.nih.gov**.

DHHS and NIH are Equal Opportunity Employers. Applications from women, minorities and persons with disabilities are strongly encouraged. NICHD/NIH is a smoke-free workplace.

Department of Health and Human Services
National Institutes of Health
National Heart, Lung, and Blood Institute

NHLBI - Cardiovascular Branch
Postdoctoral Fellowships

The National Heart, Lung and Blood Institute (NHLBI), Division of Intramural Research seeks to hire Postdoctoral Fellows to work within the Cardiovascular Branch. Areas of focus include: genomics of atherosclerosis, oxidant signaling and aging and the molecular regulation of mitochondria in cardiovascular health and disease. Research work will involve utilizing various genetic approaches on patient samples, animal disease models and in cell culture.

The postdoctoral position requires a Ph.D. and/or M.D. degree. Applicants should have experience in molecular biology and a history of prior publications in peer reviewed journals. The successful candidate will be offered stipend support commensurate with experience. Please submit a curriculum vitae, a brief statement of future research interests and future career goals, along with the names and telephone numbers, postal and e-mail addresses of three references to the Laboratory within the Cardiovascular Branch which aligns with your interest:

Web link: http://dir.nhlbi.nih.gov/labs/cb/index.asp

Toren Finkel, M.D., Ph.D.
Branch Chief — *Oxidant Signaling/Aging*
Email: **finkelt@nhlbi.nih.gov**

Paul M. Hwang, M.D., Ph.D.
Investigator — *Genomics of Atherosclerosis and Mitochondria*
Email: **hwangp@nhlbi.nih.gov**

Michael N. Sack, M.D., Ph.D.
Investigator — *Molecular Regulation of Mitochondrial Biogenesis and Metabolism*
Email: **sackm@nhlbi.nih.gov**

DHHS and NIH are Equal Opportunity Employers

Duke University
Pratt School of Engineering
Department of Biomedical Engineering

The Pratt School of Engineering is currently undergoing a period of significant growth in faculty and physical resources. As part of these expansion plans, Duke has recently launched a campus-wide strategic "Duke Bioengineering Initiative". The Pratt School of Engineering of Duke University invites applications for a faculty position in the Department of Biomedical Engineering at the Associate or Full Professor rank to lead the Bioengineering Initiative. We seek a leader who will capitalize on the interdisciplinary environment at Duke. Applications are invited from candidates with research interests in all areas of Bioengineering. Particular areas of interest include functional imaging applied to genomics and proteomics, translational bioengineering focused on regenerative medicine and drug and gene delivery, biomaterials and quantitative biology

Candidates must have a doctorate in engineering or a related field of science and an outstanding record of accomplishment. The successful candidate will be expected to have a world-class research program, initiate collaborative research with other faculty at Duke University and the Medical Center, and have a strong commitment to teaching at the undergraduate and graduate levels. This individual will play a critical role in the conception and implementation of the Duke Bioengineering initiative, including leading the recruitment of additional faculty that will be dedicated to this initiative. The newly opened Center for Interdisciplinary Engineering, Medicine and Applied Science will provide research space for the Duke Bioengineering Initiative.

Applicants must submit a curriculum vitae (with address, phone number, and e-mail address); statements of research and teaching interests; and names, addresses, phone numbers, and e-mail addresses of three references to: **Chair, Duke Bioengineering Initiative Search Committee, Department of Biomedical Engineering, Duke University, 136 Hudson Hall, Campus Box 90281, Durham, NC 27708-0281**. Applications must be received by February 15, 2005 to be given full consideration. Duke University is an Affirmative Action/Equal Opportunity Employer.

Duke University

Duke University is an Affirmative Action/Equal Opportunity Employer.

HARVARD UNIVERSITY
DIVISION OF ENGINEERING AND APPLIED SCIENCES

The Division of Engineering and Applied Sciences at Harvard University invites applications for a faculty position in Environmental Microbiology. The position is part of an initiative at Harvard in Environmental Sciences and Engineering. In addition, there are important linking opportunities with a University initiative in the Microbial Sciences and in interdisciplinary connections to the Department of Earth and Planetary Sciences. We intend to make this appointment at the Assistant or, in exceptional cases, at the Associate Professor level (untenured).

We are interested in candidates across broad areas of Environmental Microbiology, including:

- surface-/geo- microbiology and applications to environmental problems
- kinetics and interactions of microbial communities in problems of environmental engineering
- role of biofilms in environmental engineering processes
- microbial processes related to climate

Candidates are sought who have expertise in molecular chemical and biological sciences of the environment with links to the physical environment and engineering. We particularly encourage applications from women and minorities. An application should include a curriculum vitae, separate two-page statements of research and teaching interests, and up to three scientific papers. Three to five letters of recommendation should be requested and sent separately. Applications will be reviewed beginning **January 31, 2005**, although applications received after that date may also be considered. Send applications by mail or email (a single PDF file) to: **Chair, Environmental Microbiology Search Committee, Division of Engineering and Applied Sciences, 29 Oxford Street, Pierce Hall, Room 126, Harvard University, Cambridge, MA 02138; environmental_microbiology@deas.harvard.edu**.

Harvard University is an Affirmative Action/ Equal Opportunity Employer.

PET/SPECT Radiochemist Tenure Track at all ranks Radiology and Biomedical Engineering, University Hospitals, Case Western Reserve University. Successful candidate to develop an independent research program while serving as key investigator and team member in existing active programs. CASE has a campus-wide cellular and molecular imaging initiative with significant institutional and grant support. Facilities include radiochemistry labs and cyclotron, small animal PET, SPECT, CT, MR, bioluminescence, fluorescence, OCT. A significant startup package is available. Candidates should have a Ph.D. and postdoctoral research experience.

Send a statement of research and teaching interests, curriculum vitae, and the names, addresses, and phone numbers of three references to the chair of the search committee, **Raymond F. Muzic, Jr., Radiology, University Hospitals, 11100 Euclid Avenue, Cleveland, OH, USA. www.case.edu**.

CASE

CASE WESTERN RESERVE UNIVERSITY

The University is an Equal Opportunity/ Affirmative Action Employer.

Faculty Position in
Mammalian Developmental Biology
University of Illinois at Urbana-Champaign

Applications are invited for a position in mammalian developmental biology in the Department of Animal Sciences as part of a campus-wide initiative in genomic biology. This interdisciplinary initiative includes new faculty lines and the construction of the state-of-the-art $75 million Institute for Genomic Biology (IGB; **www.igb.uiuc.edu**). Research on stem cell development is of particular interest but all areas of mammalian developmental biology will be considered including reproduction (**www.life.uiuc.edu/repro**) and regenerative biology/tissue engineering (ReBTE **www.igb.uiuc.edu/research_themes/regenerative_bio.html**). The ReBTE theme will be housed in the IGB and the successful candidate will have the opportunity to contribute to this program. The position is at the **Assistant or Associate Professor** level. Applicants should have a doctoral degree, postdoctoral experience, and evidence of outstanding research potential (demonstrated research for Associate Professor). The successful candidate will establish an internationally recognized research program, direct graduate students and postdoctoral fellows, advise and interact with undergraduates, contribute to the teaching needs of the department, compete for extramural research funds, and participate in the public service mission. Excellent laboratory facilities, substantial start-up funds, and a salary commensurate with experience will be provided. Information about the position and the department can be found at **http://www.ansci.uiuc.edu/jobs/mamdev.html**.

An application must include a curriculum vitae with a complete list of publications, a concise summary of research accomplishments and future research plans, and contact information for three references. The application must be submitted in PDF format to **http://www.ansci.uiuc.edu/jobsubmission.html**. Questions should be addressed to **Dr. David Miller djmille@uiuc.edu**. To ensure full consideration, applications should be received by **February 15, 2005**.

The University of Illinois at Urbana-Champaign is an Affirmative Action, Equal Opportunity Employer.

CHIRON

Creating products that transform human health worldwide.

At Chiron, our aim is to prevent and treat diseases, and improve people's lives. A global biopharmaceutical leader with over 5500 employees worldwide, we currently seek the following professional at our headquarters in Emeryville, California:

SPECIALIST I, RESEARCH

Responsible for the expression, in prokaryotic and eukaryotic systems, of potentially therapeutic proteins and cancer associated protein targets. Duties include generation of appropriate expression constructs, initial analysis of protein expression and entry of results into a central database. Requires a minimum of 7 years of related experience with a Bachelor's degree, or minimum 5 years of experience with a Master's degree or equivalent. Molecular biology experience including the expression of heterologous proteins is required. Mammalian expression and/or high-throughput protein expression are a plus. Candidate should be highly motivated, demonstrate the ability to work independently towards a team goal, to be flexible, to show ability to act as part of a team and to work on several projects at one time.

To apply, and for more information, please visit us online, referencing job **44002902-RK**. EOE

www.chiron.com

BROAD INSTITUTE

The **Broad Institute** of **MIT** and **Harvard** seeks applications for new faculty whose research explores comprehensive approaches to biology and their application to disease.

The Broad Institute is a newly launched collaboration of MIT, Harvard, the Harvard teaching hospitals, and the Whitehead Institute for Biomedical Research. Our mission is to pursue comprehensive approaches to biological systems, with a particular focus on disease biology, enabling scientists to undertake collaborative projects that cannot be readily undertaken in more traditional academic settings. Current areas of activity include genome research in both mammalian and non-mammalian systems, medical and population genetics in human and mouse, cancer genomics, chemical biology, infectious disease, metabolic disease and psychiatric disease.

Faculty appointments at the Broad Institute are made in conjunction with a primary academic department at MIT or Harvard. The appointments include both Core Members, whose laboratories will be located primarily at the institute, and Associate Members, whose laboratories will be located primarily in their primary academic department.

We are currently seeking applicants for a tenure-track joint position as a Core Member of the Broad Institute and Assistant or Associate Professor in the MIT Department of Biology. The successful applicant will lead a world-class research program, have wide-ranging interests in comprehensive approaches to biological systems and an interest in disease biology. Applications are welcomed from scientists working in any of a variety of relevant fields (including molecular biology, genomics, medical genetics, chemistry, computational science, engineering) or at the interface of multiple disciplines, and on either human or model organisms.

Applicants should submit a curriculum vitae, a summary of current and proposed research programs, and should arrange for three letters of recommendation to be sent to: **Biology Search Committee, Attn: Professor Eric Lander, MIT Room 68-132, 77 Massachusetts Avenue, Cambridge, MA 02139.** Consideration of completed applications will begin on **January 14, 2005**.

MIT is an Affirmative Action/Equal Opportunity Employer. Qualified women and minority candidates are especially encouraged to apply.

MOUNT SINAI SCHOOL OF MEDICINE

DEPARTMENT OF MICROBIOLOGY MOUNT SINAI SCHOOL OF MEDICINE

FACULTY POSITION

VIRAL PATHOGENESIS

An Assistant/Associate Professor tenure-track position is available for candidates with research interests in the molecular and/or immunological basis of viral pathogenesis. Applicants should have a Ph.D. and/or M.D. with relevant postdoctoral experience. Candidates for Assistant Professor will be judged on their potential to develop a vigorous independent research program that can attract extramural support. Applicants to be considered for Associate Professor must have a significant publication record and extramural support. Candidates will also be expected to participate in teaching medical and graduate students as well as supervise dissertation research. An attractive start-up package and excellent facilities will be available to the successful candidate.

Submit curriculum vitae, statement of research interests and a list of three references to:

Search Committee
Department of Microbiology
Mount Sinai School of Medicine
1 Gustave Levy Place
New York, NY 10029

ryan.camping@mssm.edu

We are an Equal Opportunity/Affirmative Action Employer.

Faculty Openings in Nanoscience at Purdue University

Purdue University has several faculty openings in the broad area of nanoscience. This is part of a campus-wide emphasis on nanoscience and nanotechnology. This effort includes a new $60M interdisciplinary center for nanotechnology, and approximately 20 new faculty positions that will be added in the Schools of Engineering and Science over the next few years.

We seek exceptional faculty to complement and expand our existing expertise in all areas of experimental and theoretical nanoscience, but will give special emphasis to candidates with research interests in the areas of semiconductor nanostructures, advanced imaging at the nanometer scale especially as applied to biological systems, and computational nanoscience. Successful candidates will likely have a primary home department in either Physics, Biology, or Chemistry, and may have a joint appointment in another of these departments or in the School of Engineering at Purdue.

Candidates at all levels are encouraged to apply. Joint appointments across departments in the School of Science, or involving the Schools of Science and Engineering, are anticipated.

The department also plans to fill, in a school-wide effort, a number of physics faculty positions in multidisciplinary areas. Within this effort, the department seeks to fill positions in the areas of membrane sciences, massive data, climate change, and science education. Applicants in these fields should address the multidisciplinary contributions of their work in their research statement. For more information see **http://www.science.purdue.edu/COALESCE/**.

Purdue University is an Equal Opportunity/Equal Access/Affirmative Action Employer and is committed to building a diverse faculty of excellence. Women and minorities are especially encouraged to apply.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
OFFICE OF THE DIRECTOR
OFFICE OF SCIENCE POLICY

The Office of Science Policy in the Office of the Director, National Institutes of Health, located in Bethesda, Maryland is seeking applications for a **Health Science Policy Analyst**. The Analyst will identify emerging biomedical research and policy issues; analyze and develop policies that affect the conduct of medical research; interact with senior NIH officials, Congressional staff, and White House officials; attend, plan and coordinate national meetings to develop research agendas and policies; prepare reports and analyses of research and related social, legal and ethical issues.

Candidates should have experience and comprehensive knowledge of biomedical research and/or policy analysis; the ability to translate complex medical research concepts to lay audiences; and outstanding writing skills. A candidate with a Ph.D. or M.D. degree is highly desirable, although not required. Applications from women and minorities are strongly encouraged.

This is a civil service position with a salary range of GS-12, $60,638 to GS-14, $110,775. The vacancy announcement for this position contains complete application procedures and lists all information which should be submitted with your application. To obtain the vacancy announcement for this position which will be available on **12-1-2004** and posted under announcement **# OD-04-7857**, you may visit the NIH Career website at: **<http://careerhere.nih.gov>**. Applications may be faxed to **301-480-3063** or submitted on-line at the website listed above. Faxed information should include the vacancy number and made to the attention of **Winnie Garner**. Application and supporting information must be received by the closing date, **January 14, 2005**, in order to be considered.

DHHS and NIH are Equal Opportunity Employers

VSU
Valdosta State University

Head
Department of Biology
College of Arts and Sciences

Valdosta State University is seeking applications for a tenure-track, twelve-month position as Head of the Department of Biology at the rank of associate or full professor. Requirements include a Ph.D. in a biological field, strong teaching and research skills, and professional involvement. Administrative experience is preferred. Candidates must have the capability and desire to provide strong leadership and be committed to excellence in undergraduate and graduate education. The starting date is July 1, 2005. The salary is competitive and commensurate with qualifications and experience.

Valdosta State University, a multipurpose regional university within the University System of Georgia, has an enrollment of approximately 10,000 students. The Department of Biology includes 24 full-time faculty members and has over 500 undergraduate majors. A new Master's Degree is awaiting system approval.

Applicants should submit a letter of application; faculty application form (**http://www.valdosta.edu/academic/forms/fac_employment_app.pdf**); a current curriculum vitae; a summary statement that includes research interests, teaching philosophy, and administrative experience; copies of transcripts of all undergraduate and graduate work; and letters from at least three references. All materials and correspondence should be sent to: **Mylan Redfern, Search Committee Chair, c/o Office of the Dean, College of Arts and Sciences, Valdosta State University, Valdosta, GA, 31698**. Review of complete applications will begin **January 31, 2005**, and continue until position is filled. For more information on the University, College, Department or community visit our website **www.valdosta.edu**.

Valdosta State University is an Equal Opportunity Educational Institution.

At Merck, improving patient health isn't just what we do. It's who we are, sharing a passion for life that brings out the best in a diverse workforce. That's why Merck is recognized as one of the world's leading research-based pharmaceutical companies, while being honored by Fortune as one of the "100 Best Companies to Work for in America."

Merck has built a strong franchise in the treatment of pain with market leading products. Roughly 100 million Americans suffer from the pain of arthritis, episodes of low back pain, or chronic, debilitating headaches, all at a total cost of $100 billion in lost work and productivity. Significant medical opportunities exist for new analgesics with greater efficacy and tolerability than currently available drugs. The impact on quality of life can be substantial and mounting evidence suggests that persistent pain can result in changes in the nervous system giving rise to chronic pain long after the initial insult. Merck Research Laboratories (MRL) in **West Point, PA** is expanding its team of pain researchers to develop the next generation of breakthrough treatments.

Research Fellow – SCI002121

The incumbent will lead/initiate research programs in pain and migraine research. The ideal candidate will have 5-10 years of experience in pain and/or migraine research with solid academic qualifications (PhD, MD/PhD, MD or equivalent). Requirements include strong scientific achievements and expertise, as well as recognition for creative work in the field of pain/migraine research as evidenced by an excellent publication record. Pharmaceutical or biotechnology experience desired. Excellent leadership, interpersonal, communication, team-building and problem-solving skills are essential.

Senior Research Biologist/ Research Fellow – BIO000786

The successful candidate will be responsible for novel target identification and initiating/leading research programs. The incumbent will develop a strategic vision for pain research in order to build a rich pipeline of clinical candidates.

Requirements include a minimum of 5+ years of experience (including PhD, MD, or MD/PhD plus postdoctoral training) in molecular neuroscience with solid academic qualifications. Individual will be recognized as a leading edge thinker and would have an excellent publication record. Additional pharmaceutical or biotechnology experience is a plus. Requirements include the ability to formulate a vision, and to develop and direct strategic and scientific plans. Excellent leadership, interpersonal, communication, team-building and problem-solving skills are essential.

In return for your considerable skills, we offer an excellent salary and comprehensive benefits program, including tuition reimbursement and one of the best 401(k) plans in the nation, as well as opportunities for personal growth. We invite you to visit us online to create your profile and attach your CV at **www.merck.com/careers**.

MERCK

Committed to bringing out the best in medicine. And in people.

www.merck.com/careers
Merck is an equal opportunity employer—proudly embracing diversity in all of its manifestations.

Scripps Institution of Oceanography
Faculty Position in
Marine Invertebrate Zoology

The Scripps Institution of Oceanography of the University of California, San Diego invites applications at the Assistant, Associate, or Full Professor level (tenure track or tenured) in metazoan invertebrate zoology with a marine emphasis. Areas of interest are not restricted to a particular level of biological organization. The successful applicant will have the opportunity to assume the curatorship of the SIO Benthic Marine Invertebrate Collection if so desired. Applicants must hold a Ph.D. degree or equivalent and will be expected to teach, supervise graduate research, conduct an active research program, and participate in administrative functions of SIO and UCSD. Assistant-level applicants will be expected to show evidence of their potential by a publication record appropriate for their experience. More senior applicants must show evidence of a strong research record in their specialty. The level and salary will depend on the experience of the successful applicant and will be based on the University of California pay scale. The closing date for applications is **February 15, 2005**.

Applicants should send a letter including descriptions of their teaching and research interests, a list of publications, and names of at least five potential referees to: **Chair, SIO Graduate Department, 0208, Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA 92093-0208**.

UCSD is an Equal Opportunity Employer.

Faculty Positions in Stem Cell Research

The San Antonio Institute for Cellular and Molecular Primatology (SAICMP) in conjunction with The Department of Biology at The University of Texas at San Antonio (UTSA), and The Department of Obstetrics and Gynecology at the University of Texas Health Science Center at San Antonio (UTHSCSA) is seeking outstanding candidates for **one or two** tenured full professorships and **one or two** tenure-track/tenured assistant/associate professorships in stem cell research (pending budgetary approval). Applicants at the Full Professor level may be considered for an endowed position. The SAICMP is a newly formed institute jointly sponsored by UTSA and UTHSCSA focused on aspects of embryology, stem cell biology, epigenetics, transgenesis, biogenesis research, and regenerative medicine in nonhuman primates and other model organisms including rodents. These positions will establish a stem cell research group that will interact with many other established research groups at UTSA and UTHSCSA.

REQUIRED QUALIFICATIONS: All successful applicants will have a Doctoral degree (Ph.D., M.D. or equivalent) in Biology or a related discipline. Applicants at the **Assistant Professor** level will have relevant postdoctoral experience and demonstrated potential for excellence in stem cell research. Preference will be given to those candidates with experience working with human or nonhuman primate embryonic or gametogenic stem cells, although experience with ES or EG cell research in rodent models or with somatic stem cell research will also be considered. An applicant at the **Associate Professor** level will have all of the above plus a well-established research program with a record of extramural funding. An applicant at the **Full Professor** level will have all of the above plus a distinguished record of sustained funding and productivity as well as leadership in their field.

RESPONSIBILITIES: All positions will hold primary appointments in the Department of Biology at UTSA, with the option to hold an adjunct position at UTHSCSA. Those occupying these positions will be expected to maintain an active, externally funded research program, to participate in collaborative research activities with other members of the SAICMP, to participate in teaching at one of the two UTSA campuses or at UTHSCSA, and to participate in administrative service at UTSA.

APPLICATION: Applicants must submit, by regular mail, fax, or email, a letter of application, a current dated curriculum vitae, copies of 3 recent publications, a statement of research plans and the names, addresses (both postal and email), and telephone numbers of three references. Applicants should indicate in a cover letter the rank (assistant, associate, or full professor) of the position for which they are applying. Review of completed applications will begin immediately and will continue until the positions are filled. Address applications to: **Aaron Cassill, Ph.D., Chair, Department of Biology, The University of Texas at San Antonio, 6900 N. Loop 1604 W., San Antonio, TX 78249**. Fax applications to **(210) 458-5658** or email applications to **biofacultyad@utsa.edu**. Applicants who are not US citizens must state current visa and residency status. We seek candidates committed to our mission of mentorship serving a diverse student body. Competitive salaries and start-up packages are offered.

UTSA is an Affirmative Action/Equal Employment Opportunity Employer.
Women and minorities are encouraged to apply.

UNMC Eppley Cancer Center
Associate Director, Cancer Prevention and Control

The University of Nebraska Medical Center (UNMC) Eppley Cancer Center, a National Cancer Institute-designated Clinical Cancer Center, seeks outstanding candidates for the position of Associate Director, Cancer Control and Prevention. The position may be tenured or tenure-leading with academic rank commensurate with experience.

Applicants should have a Ph.D., M.D. or other doctoral level degree, with appropriate post-doctoral training and a track record of funding in cancer epidemiology and/or cancer prevention and control. The successful applicant will be expected to develop comprehensive, extramurally funded cancer epidemiology, cancer control and prevention research programs and to collaborate with other Cancer Center Investigators including our NCI SPORE program in pancreatic cancer and our NCI-funded Cancer Research Training Program. The Cancer Center has active multidisciplinary research programs in lymphoma, breast, prostate, pancreas, GI, and aero digestive cancers.

Resources to build population sciences research in the Cancer Center, including funds to recruit several cancer epidemiology, and cancer control and cancer prevention faculty, is expected to be part of the successful candidate's recruitment package.

Applicants should send their CV and a statement outlining their vision for the development of cancer epidemiology, and cancer and prevention programs to: **Dr. Ken Cowan, Director UNMC Eppley Cancer Center, University of Nebraska Medical Center, 986805 Nebraska Medical Center, Omaha, NE 68198-6805**. Applicants are encouraged to apply online to position **# 0013** at **https://jobs.unmc.edu**. Additional information about the UNMC Eppley Cancer Center is available at **www.unmc.edu/cancercenter/**.

Department of Health and Human Services
National Institutes of Health
National Institute of Mental Health

With nation-wide responsibility for improving the health and well being of all Americans, the Department of Health and Human Services (DHHS) oversees the biomedical research programs of the National Institutes of Health (NIH) and those of NIH s research Institutes.

The National Institute of Mental Health (NIMH), a major research component of the NIH, and the DHHS, is recruiting for a tenure-track appointment in the new Genes, Cognition and Psychosis Program under the direction of Daniel R. Weinberger, M.D. This program includes investigators from many disciplines including molecular genetics, cell biology, clinical genetics, and behavioral neuroscience. With a complementary budget and staff, the individual selected for this position will be expected to establish an independent research program focused on translational genetics related to schizophrenia and related cognitive dysfunction. This will include discovery of specific genetic variants that increase risk for schizophrenia, understanding mechanisms by which these variants act, developing intermediate phenotypic measures, acquiring new subject data sets, and developing new therapeutic approaches based on these discoveries. The opportunity exists for this position to be a joint appointment with NIMH and with the National Human Genome Research Institute (NHGRI).

The successful individual must possess an M.D. and/or Ph.D. degree, and experience in a relevant area of clinical and translational genetics as well as neuroscience. At least five years of relevant research experience is required.

Salary is commensurate with experience and accomplishments, and a full Civil Service package of benefits (including retirement, health, life, and long term care insurance, Thrift Savings Plan participation, etc.) is available.

The strong scientific environment and outstanding equipment resources at NIH makes this a unique opportunity for an outstanding scientist. Interested candidates should send curriculum vitae, statement of research interests, accomplishments and future goals, and six letters of recommendation to the **Chair, Search Committee for a Tenure Track Investigator in the area of Translational Genetics, National Institute of Mental Health, Building 10, Room 4N-222, 9000 Rockville Pike, Bethesda, MD 20892**, or by email to: **steyerm@mail.nih.gov** by **January 24, 2005**.

DHHS and NIH are Equal Opportunity Employers

AAAS Annual Meeting and *Science* Career Fair

The 2005 AAAS Annual Meeting is the perfect place to explore your career options and attend the *Science* Career Fair. Both the career fair and the career-related workshops are FREE to attend.

AAAS Annual Meeting

DATES: **17–21 February 2005**

PLACE: **Marriott Wardman Park Hotel Washington, DC**

CAREER WORKSHOPS (see website for complete listing of workshops):

- Strategic Networking
- Pathways to Multiple Career Opportunities
- AAAS Fellowship Program in Public Policy and Mass Media
- Research Training at the NIH
- How to Fire Up your Presentation

AAAS/*Science* Career Fair

DATE: **21 February 2005**

PLACE: **Marriott Wardman Park Hotel Washington, DC**

TIME: **11:00 am – 4:00 pm**

Science Careers offers you the chance to meet employers.

Exhibiting employers are typically from biotechnology, pharmaceutical, government, and manufacturing organizations.

For more information and updates to our exhibitor list, please visit www.sciencecareers.org and click on Career Fairs.

▶ **Registration is required to attend the career workshops. Visit www.sciencecareers.org and click on Career Fairs for instructions on how to register for free.**

ScienceCareers.org

We know science AAAS

POSITIONS OPEN

PROBATIONARY FACULTY POSITIONS (2005-2006)
Department of Biological Sciences
St. Cloud State University
St. Cloud, Minnesota

Community Ecology

Teach ecology, community ecology, and mammalogy. Shared responsibilities in introductory biology for majors and nonmajors and graduate courses. Active research program with an emphasis in plant-animal interactions.

Genetics/Molecular Biology

Teach genetics and advanced DNA techniques courses. Shared responsibilities in other undergraduate and graduate courses that complement existing faculty expertise. An active research program employing a functional genomics or whole genome approaches to basic biological questions is preferred.

Qualifications: Ph.D. in relevant field of biological sciences required. Postdoctoral or equivalent experience required. Preference given to candidates with successful post-secondary teaching experience and a strong commitment to undergraduate instruction. A research program involving undergraduate and Master's graduate students and experience compatible with current faculty interests and expertise is expected. Advising and committee participation are expected. The successful candidate will be required to document the following for promotion and tenure: ability to teach and/or perform effectively, scholarly achievement or research, continued preparation and study, contribution to student growth and development, and service to the University and community. The successful candidate will have demonstrated ability to teach and work with people from culturally diverse backgrounds.

Send: letter of application including research plans, teaching philosophy, curriculum vitae, transcripts (copies acceptable for initial screening), and the name, telephone number, and postal and e-mail address of three references. We will contact references to comment specifically upon your teaching ability, experience, and professional preparation. Submit materials to: **Chair, Department of Biological Sciences, St. Cloud State University, 720 4th Avenue South, MS-262, St. Cloud, MN 56301-4498.** You may contact us by **telephone: 320-308-5433; fax: 320-308-4166;** or **e-mail: biology@stcloudstate.edu** or **website: http://www.StCloudState.edu/~biol.** All materials must be received by January 18, 2005, to be considered. *St. Cloud State University is committed to excellence and actively supports cultural diversity. To promote this endeavor, we invite individuals who contribute to such diversity to apply, including minorities, women, gays, lesbians, bisexuals, transgenders, persons with disabilities, and veterans.*

ECOLOGIST
University of North Texas

The Department of Biological Sciences at the University of North Texas (UNT) invites applications for a **TENURE-TRACK POSITION** in environmental science. Expertise in understanding responses to environmental perturbations at the cellular, organismal, community, or ecosystem level is desired. Wetlands (plant) ecologists or aquatic toxicologists are especially encouraged to apply. Application review will begin on February 15, 2005, and will remain open until filled. For further information on the Environmental Science Program, visit **websites: http://www.biol.unt.edu** and **http://www.ias.unt.edu.**

Applicants should send letter of interest and future research directions, curriculum vitae, names and contact information for at least three references, and up to three reprints or manuscripts to: **Dr. Thomas W. La Point, Chair, Environmental Science Search Committee, University of North Texas, P.O. Box 310559, Denton, TX 76203-0559.**

UNT is an Affirmative Action/Equal Employment Opportunity Institution committed to diversity in its employment and educational programs, thereby creating a welcoming environment for everyone.

POSITIONS OPEN

TENURE-TRACK FACULTY POSITION
University of Wyoming
Department of Molecular Biology

The Department of Molecular Biology (**website: http://www.uwyo.edu/MolecBio/**) is seeking to fill a research-intensive tenure-track position at the **ASSISTANT PROFESSOR** level. Outstanding candidates are sought who study important biological problems using the tools of computationally intensive molecular biology broadly defined as bioinformatics, modeling, genomics, proteomics, nuclear magnetic resonance (NMR) spectroscopy, or related specialties. Candidates for this position must have an earned Ph.D. degree, at least two years of postdoctoral training, and a strong publication record. Successful candidates will be expected to develop independent extramurally funded research programs. The candidate will also participate in the departmental teaching mission, which involves undergraduate, M.S./Ph.D., and medical students. Competitive salary support, startup funds, and renovated laboratory space will be provided.

The Department is presently composed of 12 faculty members with diverse research interests in biochemistry, biophysics, genetics, and microbiology supported by over $7.5 million per year in grants. The University has a 700 MHz NMR spectrometer, a 50-node UNIX cluster for biological calculations, electrospray, and matrix-assisted laser desorption/ionization – time of flight mass spectrometers, a Jasco circular dichroism spectrometer, as well as a state-of-the-art facility for imaging including confocal microscopy, scanning electron microscopy, transmission electron microscopy, and atomic force microscopy. The University is located in the Rocky Mountains about 120 miles northwest of Denver, Colorado, and enrolls 12,000 students, including approximately 2,200 graduate students.

Applications should include a cover letter, curriculum vitae, statement of research interests, statement of teaching interests, and copies of key publications. Send application materials in PDF format and inquiries to **e-mail: ahrenhol@uwyo.edu.** Arrange for three letters of reference to be sent directly to: **Faculty Search Committee, Department of Molecular Biology, Department 3944, University of Wyoming, 1000 E. University Avenue, Laramie, WY 82071.** Review of applicants will begin on February 1, 2005, and continue until a suitable candidate has been identified.

The University of Wyoming is an Affirmative Action/Equal Opportunity Employer.

The Institute of Atomic and Molecular Sciences, Academia Sinica, invites qualified candidates to apply for **TENURE-TRACK RESEARCH FELLOW POSITIONS** in the following research fields: experimental atomic physics, surface science, biophysical science, nano-science, theoretical molecular dynamics, and ultrafast and high-field optics. Please visit **website: http://www.iams.sinica.edu.tw** for detailed academia activities of the Institute. Successful candidates must show excellent academic achievements and abilities to establish a successful research program within the Institute in the above research fields. Collaborations with other research fellows are strongly encouraged. Interested applicants should send full curriculum vitae by airmail, including a list of publications, a research proposal, and at least three letters of recommendation to: **Dr. Szu-yuan Chen, Room 325, P.O. Box 23-166, Institute of Atomic and Molecular Sciences, Academia Sinica, Taipei, Taiwan. E-mail: sychen@ltl.iams.sinica.edu.tw; fax: 886-2-2362-7064.** To ensure timely processing, all application materials must be received by February 28, 2005.

MEDICAL WRITER PHARMACEUTICALS

Strong general biomedical background; flair for writing; ability to evaluate scientific literature and learn new subjects. Onsite, write journal articles and product information. Resume to: **Project House, 433 Hackensack Avenue, 4th Floor, Hackensack, NJ 07601. E-mail: projecthouse@projhouse.com.**

POSITIONS OPEN

ASSISTANT PROFESSOR
Marine Science
(Physical Oceanography)

Coastal Carolina University (CCU) invites applications for a Tenure-track Assistant Professor position in the Department of Marine Science beginning in August 2005. Applicants must have a Ph.D. in physical oceanography or related discipline, excellent communication skills, demonstrated instructional abilities, and a strong commitment to undergraduate interdisciplinary education. We particularly seek applicants with research experience that includes coastal ocean processes and interdisciplinary collaboration. The successful candidate will be expected to teach a core physical oceanography course, develop upper-division and graduate courses consistent with the Coastal Marine and Wetlands Study M.S. program, and develop a successful field-oriented research program involving both undergraduate and graduate students.

Coastal Carolina University is a growing, state-supported liberal arts institution where the emphasis is on undergraduate education, and growing importance is placed on faculty mentored student research projects and public services. Coastal Carolina University is located approximately nine miles from Myrtle Beach, South Carolina, and enrolls more than 7,000 students.

Review of applications will begin February 1, 2005, and continue until the position is filled. Please send curriculum vitae, statement of teaching and research expertise, selected reprints, and three letters of recommendation to: **Chair, Coastal Carolina University, Department of Marine Science, P.O. Box 261954, Conway, SC 29528-6054.** For further information about CCU and Marine Science visit **website: http://kingfish.coastal.edu/marine.**

Coastal Carolina University is an Equal Opportunity/Affirmative Action Employer.

FACULTY POSITION
Department of Bioscience
School of Science and Technology
Kwansei Gakuin University, Japan

A faculty position is available in bioscience at the **PROFESSOR, ASSOCIATE,** or **ASSISTANT PROFESSOR** level. The candidate must have a Ph.D. or M.D. degree and strong expertise in the field of molecular biology (preferably in plant biology). The incumbent is expected to assume the assignment on April 1, 2006.

Candidates should send by March 31, 2005, (a) curriculum vitae (with a recent photo); (b) list of publications; (c) reprints of no more than three papers; (d) summary of past research and future research plans (no more than 1,500 words); (e) two reference letters (or the names, addresses, and contact information of two references) to: **Professor Yaichi Shinohara, Dean, School of Science and Technology, Kwansei Gakuin University, 2-1 Gakuen, Sanda, Hyogo 669-1337, Japan.** Contact: **Professor Hiroshi Yamasaki. Telephone: 81-79-565-8734; e-mail: hyamasaki@ksc.kwansei.ac.jp.**

INVERTEBRATE ZOOLOGIST, East Tennessee State University (ETSU) Biological Sciences. Tenure-track, nine-month **ASSISTANT PROFESSOR** beginning August 15, 2005. Responsibilities include teaching introductory biology, invertebrate zoology, and upper-level/graduate courses in area of specialization. Ph.D. required by start date. Expectations include development of extramurally funded research involving B.S. and M.S. students. Preference given to applicants with postdoctoral experience, expertise in community ecology, and potential for research in Appalachia. More information available at **website: http://www.etsu.edu/biology.** Send letter of application, curriculum vitae, statements of research plans and teaching philosophy, and three letters of reference. Electronic submission preferred to: **Dr. Rebecca Pyles** at **e-mail: pylesr@etsu.edu.** Application reviews begin February 1, 2005, and continue until position is filled. *ETSU is an Affirmative Action/Equal Opportunity Employer.*

FACULTY POSITION IN FOOD SAFETY AND TECHNOLOGY ILLINOIS INSTITUTE OF TECHNOLOGY – CHICAGO

The National Center for Food Safety and Technology (NCFST, www.ncfst.iit.edu) and the Biological, Chemical and Physical Sciences (BCPS) Department at Illinois Institute of Technology (IIT, www.iit.edu) invite applications for a tenure-track Assistant Professor position with a joint appointment. Exceptional candidates in any field of food safety and technology may apply; however, we particularly encourage candidates with an interest in microbiology who use proteomics, genomics, microarray technology or other molecular approaches to study problems related to microbial stress responses. A Ph.D. degree and post-doctoral experience are required. The successful candidate will be provided with a competitive start-up package, and will be expected to establish an extramurally funded research program, teach in our undergraduate and graduate programs, and serve as a research advisor to graduate students. Duties will be shared between NCFST on IIT's Moffett Campus and BCPS on the main campus.

For full consideration, send a C.V, a summary of research plans, a statement of teaching interests and philosophies, and contact information for three references to:

Dr. Martin Cole, Director
National Center for Food Safety and Technology
6502 S. Archer Rd.
Summit-Argo, IL 60501

Electronic transmission of documents to **tripodi@iit.edu** is preferred. Screening of applications will begin **January 31, 2005** and will continue until the position is filled.

IIT is an Affirmative Action/Equal Opportunity Employer, and especially encourages applications from qualified women and minority candidates.

Department of Health and Human Services National Institutes of Health National Institute on Alcohol Abuse and Alcoholism

NIAAA
National Institute on Alcohol Abuse and Alcoholism

Position in Metabolomics, Laboratory of Metabolic Control, NIAAA, NIH, Rockville MD, USA

A position is available for scientist or technician with experience and interest in developing methods of mass spec analysis of small molecules from biological samples. Salaries range from $38,000 to $140,000 per year depending upon qualifications. Applications must be received by the closing date of **January 30, 2005**.

Interested candidates should submit their CV and bibliography to:

Dr. Richard Veech
Lab of Metabolic Control
NIAAA/NIH
5625 Fishers Lane
Rm. 2S-28, Bethesda, MD 20892-9409
FAX 301 443 0930
Email: rveech@mail.nih.gov

DHHS and NIH are Equal Opportunity Employers. Applications from women, minorities, and persons with disabilities are encouraged. The DICBR/NIAAA is a smoke-free environment.

FACULTY RECRUITMENT IN STEM CELL RESEARCH

UNIVERSITY OF CALIFORNIA, IRVINE

The University of California, Irvine, invites applications at all levels for a cluster of new tenure-track faculty positions in the area of human stem-cell research. Space designated for stem-cell research, which will be free of NIH-imposed constraints on the use of embryonic stem cells, will be available. Applicants should have a Ph.D. and/or M.D. degree and have demonstrated creativity and excellence in stem-cell research or a related area. They will be expected to complement existing strength in stem-cell research at UCI, and to take advantage of major new funding opportunities from state as well as federal and private sources. Those with a track record showing potential leadership in such an endeavor are strongly encouraged to apply. Appropriate areas of expertise include, but are not restricted to, potential new therapeutic applications of stem cells studied in animal models and/or human patients, as well as basic research on stem cell properties including development, proliferation, migration, and plasticity. Successful applicants will also be expected to develop and teach new courses at the undergraduate and graduate levels. The level of appointment is flexible and will depend on the applicant's qualifications.

Applicants should send a complete CV, a selection of reprints (five or less), and a brief statement of future research plans and teaching interests. They should arrange for three reference letters to be sent to: **Stem Cell Search Committee of the School of Biological Sciences and College of Medicine, BSA 100, University of California, Irvine, Irvine, CA 92697**. Application review will begin **January 3, 2005**, and will continue until the positions are filled.

The University of California, Irvine has an active career partner program and a National Science Foundation Advance Gender Equity Program and is an Equal Opportunity Employer committed to excellence through diversity.

Faculty Positions in Membrane Science at Purdue University

The School of Science at Purdue University has several faculty positions available in the area of **Membrane Science**. Applications are being solicited from dynamic individuals with an interest in studying the structure and function of biological membranes and membrane proteins. In particular, candidates with a research program in the areas of structural biology or biophysical analysis of membrane proteins, genetics or cell biology of vesicle trafficking, and molecular transport are of particular interest. Candidates at all levels are encouraged to apply.

These hires are part of a campus-wide strategic hiring process that will add up to 50 new faculty in the School of Science. New faculty hires will also complement and extend a strategic hiring process in Tissue and Cellular Engineering. Faculty participants will benefit from new developments in Discovery Park at Purdue (**www.purdue.edu/DiscoveryPark/**), including construction of the Bindley Bioscience Center and the Birck Nanotechnology Center. The successful applicant could have a full or partial appointment in any of the following departments within the School of Science: Biological Sciences, Chemistry, or Physics.

For additional information about these strategic hires, the School of Science at Purdue, and how to apply, please visit our Web site at: **www.science.purdue.edu/COALESCE/**. Applicants should submit a resume, summary of research and teaching interests, and arrange to have three letters of reference sent to the same address. Application review will begin **December 15, 2005** and will continue until the available positions are filled.

Purdue University is an Equal Opportunity/Equal Access/ Affirmative Action Employer.

POSITIONS OPEN

ASSISTANT PROFESSOR NEUROSCIENCE

Ursinus College seeks to fill an Assistant Professor tenure-track position in the Neuroscience Interdisciplinary Program and the Psychology Department for fall 2005. Desire a broadly trained Neuroscientist; appropriate backgrounds include behavioral neuroscience, neurobiology, cognitive psychology, or related fields. Applicants should have a strong commitment to undergraduate teaching and research involving students in a liberal arts college. The successful candidate will be expected to teach psychology at the introductory level, neuroscience at the introductory and advanced undergraduate levels, and an interdisciplinary freshman seminar, as well as establish an ongoing research program. Previous grant-writing experience is desirable, but not essential; candidate should have the potential for external research/contract funding.

Ursinus College is a highly selective, nationally ranked, independent, co-educational residential liberal arts college of 1,450 students located about 25 miles from center city Philadelphia. Applicants should arrange for the following to be sent: letter of application, curriculum vitae, copies of relevant papers, evidence of teaching effectiveness, transcripts, and three current confidential letters of recommendation to: **Catherine Chambliss, Chair, Neuroscience Search Committee, Ursinus College, Collegeville, PA 19426-1000.** Materials should arrive by January 10, 2005. Applications received after the deadline will be considered if the position is still open. *Ursinus College is an Equal Employment Opportunity/Affirmative Action Employer. In keeping with the College's historic commitment to equality, women and minorities are especially encouraged to apply.*

FACULTY POSITION
University of Illinois at Chicago

The Department of Pharmacology at The University of Illinois College of Medicine at Chicago is seeking candidates for **ASSOCIATE PROFESSOR** or **PROFESSOR** appointment. The position would be conjoint with the Center for Lung and Vascular Biology. Candidates should have Ph.D. and/or M.D. degree, outstanding publication record, and NIH-funded research program in one of following areas: leukocyte activation mechanisms, inflammation, vascular biology, and adhesion molecules. Preference will be given to candidates who have successfully established disease models for research that complements ongoing activities including vascular biology, adhesion of leukocytes and endothelial cells, and G protein signaling in blood and vascular cells. Individuals with other relevant interests are also encouraged to apply. The successful candidate will be offered a highly competitive startup package and new research space. Please send curriculum vitae, statement of research interests/plans, and names of three references to: **Dr. Richard Ye, Search Committee A/F, Department of Pharmacology, M/C 868, University of Illinois, 835 South Wolcott Avenue, Chicago, IL 60612.** For fullest consideration, applications should be received by February 28, 2005. *The University of Illinois is an Affirmative Action/Equal Opportunity Employer.*

BIOCHEMISTRY TENURE-TRACK POSITION at the **ASSISTANT PROFESSOR** level is available fall 2005 at the California State University, San Marcos (CSUSM), contingent upon funding. Candidates must have a Ph.D. in biochemistry with postdoctoral experience. Applicants should submit a letter of application, curriculum vitae, three letters of recommendation, a one-page statement of teaching philosophy, and a two-page outline of future research plans to: **Chemistry Search Committee, California State University San Marcos, San Marcos, CA 92096. E-mail: rgrant@csusm.edu.** Review of applications begins February 15, 2005; position open until filled. *CSUSM is an Equal Opportunity/Title IX Employer. The University has a strong commitment to the principles of diversity and, in that spirit, seeks a broad spectrum of candidates including women, members of minority groups, and people with disabilities.*

POSITIONS OPEN

STONY BROOK
STATE UNIVERSITY OF NEW YORK

Stony Brook University's Department of Pharmacology seeks a **RESEARCH SCIENTIST** position. Responsibilities: Study atherosclerosis and Apo E signaling. Required: M.D. or Ph.D. in biological science, three years experience with at least one at the postdoctoral level, in morphology/histology at the light and electron microscope level, preferably with experience in immunolocalization of antigens and RNA expression analysis. Mechanisms by which low levels of plasma Apo E suppresses atherosclerotic lesion formation will be explored. Send curriculum vitae, letters of references, and of interest to: **Drs. F. Thorngate and C. C. Malbon, c/o (e-mail: brockner@pharm.sunysb.edu) Stony Brook University, Stony Brook, NY 11794-8651.** Visit **website: http://www.stonybrook.edu/cjo** for employment information. *Affirmative Action/Equal Opportunity Employer.*

BOTANIST: Eckerd College seeks a Botanist to fill a **VISITING ASSISTANT PROFESSOR** position in our biology program to begin fall 2005. The position is for two years with a possible extension to a third year. Applicants should have a Ph.D.; teaching experience is preferred. The successful candidate should be prepared to teach courses in introductory botany for majors, a science course for nonmajors, and possibly an elective course in their area of expertise. Applicants with research programs conducive to participation by undergraduates are encouraged to apply. Interested individuals should send curriculum vitae, statement of teaching and research interests, and two letters of reference by 15 February 2005 to: **Dr. Peter Meylan, Natural Sciences, Eckerd College, 4200 54th Avenue South, St. Petersburg, FL 33711.** Eckerd College is an outstanding college of the liberal arts and sciences located on Tampa Bay in St. Petersburg, Florida. More information about the natural sciences at Eckerd College can be found at **website: http://www.eckerd.edu/academics/nas/.** *Equal Opportunity Employer/Minorities/Females/Veterans/ADA.*

RESEARCH FACULTY
Division of Quality Health Care

The Division of Quality Health Care, Department of Internal Medicine at Virginia Commonwealth University is seeking a mid-level physician scientist with research expertise in shared decision-making for prostate cancer. Candidate will also have a joint appointment in Massey Cancer Center. Candidate will enhance the Division's existing research program, teach in research training programs, and maintain a modest clinical practice. Must be BC in internal medicine, have a Master's degree in clinical research, public health, or equivalent. A track record of external funding is required. Projected starting date is February 1, 2005.

Send or fax curriculum vitae and two letters of reference to: **Wally R. Smith, M.D., Chairman, Division of Quality Health Care, P.O. Box 980306, Richmond, VA 23298-0306. Fax: 804-828-4862.** *Virginia Commonwealth University is an Equal Opportunity/Affirmative Action Employer. Women, persons with disabilities, and minorities are encouraged to apply.*

Illinois Wesleyan University seeks an **INTRODUCTORY BIOLOGY LABORATORY COORDINATOR** for a full-time, nontenure-track staff appointment to start fall 2005. The successful candidate will coordinate, develop, and teach introductory biology laboratories. A Ph.D. is preferred. Review of applications will begin immediately and continue until the position is filled. Send curriculum vitae, undergraduate and graduate transcripts, statement of teaching philosophy, and three letters of recommendation to: **R. Given Harper, Chair, Department of Biology, Illinois Wesleyan University, P.O. Box 2900, Bloomington, IL 61702. E-mail: gharper@iwu.edu.** For further information see our jobs **website: http://www.iwu.edu/~iwujobs.**

POSITIONS OPEN

HEALTH SCIENCE OFFICER
U.S. Department of Veterans Affairs
Biomedical laboratory Research and Development Service

The VA's Biomedical Laboratory Research and Development Service is recruiting for a full-time Health Science Officer to manage the Agency's basic science and preclinical oncology research program. The Health Science Officer will be responsible for portfolio management and administration of VA research related to oncology and will assist with the proposal review process. Ideal candidates will have a Ph.D. degree with a research background in a relevant area. Experience in grant writing and/or review is desirable. The position is located at VA Central Office, Washington, D.C.

Closing date: December 31, 2004. To view full vacancy announcement, go to following site and type in AR152609 in keyword search. **Website: http://jobsearch.usajobs.opm.gov/.**

Follow instructions in the announcement or contact the OPM office at: **Office of Personnel Management, Raleigh Service Center, 4407 Bland Road, Suite 200, Raleigh, NC 27609. Telephone: 919-790-2864**. *Equal Opportunity Employer.*

RESEARCH ASSOCIATE POSITION
Bioinformatics, Microbial Genomics, and Biodefense
University of Alabama at Birmingham

A Research Associate position is available immediately to participate in research focused on the development of microbial databases and analytical tools to study the pathogenesis of microorganisms with an emphasis on developing bioinformatic resources to aid current biodefense research initiatives. Candidates with a Ph.D. or equivalent in either the biological or computer sciences, and experience in bioinformatics, microbial genomics, or microbiological research will be considered.

Interested candidates should apply to: **Elliot J. Lefkowitz, Ph.D., Department of Microbiology, The University of Alabama at Birmingham, BBRB 276/11, 1530 3rd Avenue S, Birmingham, AL 35294-2170. E-mail: elliotl@uab.edu. Website: http://www.genome.uab.edu.** Application deadline: February 28, 2004. *University of Alabama at Birmingham is an Affirmative Action/Equal Opportunity Employer.*

VISITING ASSISTANT PROFESSOR OF NEUROSCIENCE

Oberlin College invites applications for a one-year, noncontinuing position beginning July 1, 2005. Incumbent will teach two upper-level courses for majors and a laboratory with one of the courses and he or she will also teach two sections of an introductory neuroscience laboratory. Applicants with training in any area of neuroscience should apply, but candidates with training and interests in neuroanatomy, animal behavior, cognitive neuroscience, or systems neuroscience are particularly encouraged. Requirements: Ph.D. (in-hand or by July 1, 2005) and demonstrated interest and potential excellence in undergraduate teaching. Send letter of application, curriculum vitae, graduate academic transcripts, and at least three letters of reference to: **Dennison Smith, Chair, Neuroscience Department, 119 Woodland Avenue, Oberlin, OH 44074** by February 15, 2005. Late applications may be accepted until position filled. *Affirmative Action/Equal Opportunity Employer.*

RESEARCH GENETICIST to research and improve existing varieties of egg-type chicken using modern quantitative genetics. Ph.D. in animal science or animal genetics or related field. Three years related experience, including experience maximum likelihood method and best linear unbiased predictor. Theoretical knowledge of or practical experience with chickens and genetics. Demonstrated ability to use SAS and Unix. Send resumes to: **Thomas Jorgensen, 1775 West Lakes Parkway, West Des Moines, IA 50266.** *Equal Opportunity Employer.*

COURSE

COURSE ANNOUNCEMENT

20th Annual Offering of Critical Issues in Tumor Microcirculation, Angiogenesis and Metastasis: Biological Significance and Clinical Relevance

A Continuing Education Course of Harvard Medical School and Massachusetts General Hospital Boston, MA, USA - June 6 -9, 2005

Dr. Rakesh K. Jain of Harvard Medical School and Massachusetts General Hospital is offering a Continuing Medical Education summer course entitled "Critical Issues in Tumor Microcirculation, Angiogenesis and Metastasis: Biological Significance and Clinical Relevance." The purpose of the course is to present the latest findings in systems biology of cancer.

Faculty Includes:
Peter Carmeliet, M.D., Ph.D.
Harold F. Dvorak, M.D.
Isaiah J. Fidler D.V.M., Ph.D.
Judah Folkman, M.D.
Rakesh K. Jain, Ph.D.
Robert S. Kerbel, Ph.D.

This course meets the criteria for 22 credit hours in category I of the Physician's Recognition Award of the American Medical Association.

To register or view course information online, please visit the HMS-CME home page **http://cme.med.harvard.edu**.

For more information, please access our Website: **http://steele.mgh.harvard.edu**

MRC Medical Research Council

MRC Laboratory of Molecular Biology, Cambridge, UK

Research Assistant in Neurobiology, Ref: NB/1104/15

Applications are invited for a Research Assistant position in the group of Dr Michel Goedert to work on the abnormal filamentous deposits that characterise common neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease. You should have a degree in Biochemistry or a related field, at least three years experience in protein chemistry, and an interest in the mechanisms underlying neurodegeneration. Particularly important is expertise in protein purification and analysis. Experience in electron microscopic techniques would be an advantage.

Further information about the position may be obtained from Dr Michel Goedert, e-mail: mg@mrc-lmb.cam.ac.uk

This is an open-ended position and is available from January 2005. The starting salary is likely to be in the range of £25,647 - £27,805 per annum, depending upon qualifications and experience. This is supported by a flexible pay and reward policy, and optional MRC final salary Pension Scheme. We can offer 30 days annual leave entitlement and excellent on-site sports and social facilities. Additional information about the Laboratory may be obtained at http://www.mrc-lmb.cam.ac.uk

Please quote job reference NB/1104/15, and include a covering letter and CV with the names and addresses of two professional referees who can be contacted prior to interview.

E-mail your application to: recruit@mrc-centre.cam.ac.uk or post to: Miss Kelly Andrews, Personnel Assistant, MRC Centre, Hills Road, Cambridge, CB2 2QH, UK.

Closing date: 7 January 2005

The Medical Research Council is an Equal Opportunities Employer.
'Leading Science for Better Health'

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AAAS

Post-Doctoral Positions, Rocky Mountain Laboratories, Hamilton, Montana

Starting salary $37,100 to $49,800: Commensurate with Experience/Education

DEPARTMENT OF HEALTH AND HUMAN SERVICES/NATIONAL INSTITUTES OF HEALTH. Postdoctoral positions are available to study mechanisms of staphylococcal pathogenesis and the interaction of staphylococci with the human innate immune system. Studies will include proteomics and genomics strategies to identify proteins of interest in staphylococci during interaction with human leukocytes, and during infection in relevant animal models. Candidates should have a strong background in molecular biology and microbiology with experience in cloning and targeted prokaryotic gene knock-outs. Experience with *Staphylococcus aureus* or *S. epidermidis* is desirable. State-of-the-art genomics, proteomics, DNA microarray, microscopy, and sequencing facilities are available to help identify and characterize molecules important in staphylococcal pathogenesis. The Rocky Mountain Laboratories' research campus includes an excellent electron microscopy facility, and new Biosafety Level 3 and non-human primate facilities. The laboratories are located in a very scenic mountainous area, which supports skiing, climbing, hiking, cycling, and trout fishing. A Ph.D. or M.D. in an appropriate discipline is required (with 5 or fewer yrs relevant post-doctoral experience; excluding 2 yrs clinical work for M.D.s).

Please send CV, 1-2-page career summary/research interests, and names of 3 references to:

Michael Otto, Ph.D. (motto@niaid.nih.gov)
molecular biology, *S. epidermidis* pathogenesis
or
Frank R. DeLeo, Ph.D. (fdeleo@niaid.nih.gov)
neutrophil biology, *S. aureus* pathogenesis
Rocky Mountain Laboratories
Laboratory of Human Bacterial Pathogenesis
NIAID, NIH
903 South 4th Street
Hamilton, MT 59840

DHHS and NIH are Equal Opportunity Employers.

POSITIONS OPEN

FACULTY POSITION IMMUNOLOGY/PROTEOMICS

The Integrated Department of Immunology at the University of Colorado Health Sciences Center and National Jewish Medical and Research Center (NJMRC) invites applications for a faculty position at any level. Individuals with training, experience, and interest in using proteomics approaches in the context of immunology or related disciplines are encouraged to apply. The successful candidate will develop an independent research program as a member of a highly interactive and diverse group of basic immunologists. Interactions with clinical scientists focused on asthma and chronic obstructive pulmonary disease (COPD) and with faculty of the University of Colorado Health Sciences Center Proteomics Core will be encouraged. Funds are available to establish a state-of-the-art proteomics facility on the NJMRC campus. Candidates should have a Ph.D. and/or M.D. degree, at least three years of postdoctoral experience, and a record of outstanding research. Interested individuals should send their curriculum vitae, statement of research interests, and names of three references to:

Dr. James Hagman
Chair, Search Committee
c/o Kathy Davis
National Jewish Medical and Research Center
1400 Jackson Street, K520
Denver, CO 80206
E-mail: davisk@njc.org

The University of Colorado and National Jewish Medical and Research Center are Affirmative Action/Equal Opportunity Employers.

Haskell Laboratory for Health and Environmental Sciences is seeking a highly motivated **POSTDOCTORAL RESEARCH ASSOCIATE** to develop cell culture models for hepatotoxicity. The project will address basic science issues around development of culture conditions that promote hepatotypic morphology and differentiated function of primary hepatocytes in 2-D culture, and the use of functional and molecular endpoints to assess potential of xenobiotics to produce hepatotoxicity in vivo. Haskell Laboratory has excellent facilities for biochemical and molecular analysis, cell culture, mass spectrometry, and microscopy. Qualified candidates will have a Ph.D. or equivalent in toxicology, biochemistry, physiology, pharmacology, or a related area. Experience in cell culture and biochemical and molecular analysis is essential. The successful candidate will be able to communicate research results clearly and effectively through oral presentations and publications. DuPont offers a competitive salary and excellent benefits package. For prompt consideration, please send your resume to: **P.O. Box 90, Elk Mills, MD 21920.** *Equal Opportunity/Affirmative Action Employer.*

ASSISTANT PROFESSOR/ASSISTANT SCIENTIST-Plant Bacteriology. Washington State University, Pullman, Washington. The Department of Plant Pathology, College of Agricultural, Human, and Natural Resource Sciences, invites applications for a permanent, 12-month, tenure-track position (65 percent research, 35 percent teaching). Required: earned Ph.D. in plant pathology, plant bacteriology, or related field; evidence of scholarly contributions. For a complete notice of vacancy listing qualification for this position, visit **website: http://www.hrs.wsu.edu/employment/FAPvacancies.asp** (Search # 3896). Letter of application specifically addressing each required and desired qualification, a one-page description of teaching philosophy, curriculum vitae, copies of college/university transcripts, and names and addresses of at least four professional references should be sent to: **Dr. Hanu R. Pappu, Department of Plant Pathology, P.O. Box 646430, Washington State University, Pullman, WA 99164-6430; fax: 509-335-9581; e-mail: hrp@wsu.edu.** Screening: February 25, 2005. *Equal Employment Opportunity/Affirmative Action/ADA.*

AQUACULTURE BIOLOGIST

The School of Marine Sciences at The University of Maine invites applications for an Aquaculture Biologist. This is a tenure-track, academic year position, available 1 September 2005. Rank and salary are negotiable depending upon qualifications and outstanding candidates at all levels will be considered. We are seeking an individual with expertise in physiology, pathology, parasitology, ecology, or production-related research, preferably working with shellfish. The School of Marine Sciences was formed eight years ago to foster interdisciplinary collaboration among over 40 faculty representing various subdisciplines of marine science, including aquaculture, marine biology, marine policy, and oceanography. The successful candidate will contribute to this vision and the growth of the aquaculture industry in Maine by developing a strong, externally funded research program and will have an opportunity to play an integral role in the development of an aquaculture institute at The University of Maine. The successful applicant will also be expected to contribute to the undergraduate and graduate teaching mission of the School. A Ph.D. or equivalent degree in a relevant field and postdoctoral research experience are required. Send cover letter, curriculum vitae, statements of research interests and teaching philosophy, reprints, and the names and addresses of three references to: **Chair, Aquaculture Biologist Search Committee, School of Marine Sciences, 5706 Aubert Hall, University of Maine, Orono, ME 04469-5706.** Information on the School of Marine Sciences can be found at **website: http://www.marine.maine.edu** and inquiries may be addressed to **e-mail: aqbiosearch@maine.edu**. Review of applications will begin February 1, 2005, and continue until the position is filled. *Women and minorities are encouraged to apply. The University of Maine is an Equal Opportunity/Affirmative Action Employer.*

BEHAVIORAL PHARMACOLOGIST (ASSISTANT PROFESSOR)
Department of Psychiatry

The Department of Psychiatry at The University of Chicago is recruiting faculty to implement a new phase of growth and development. At this time we are seeking a Ph.D. basic scientist for a tenure-track position to develop a basic behavioral pharmacology program in the mechanism of action of psychotropic agents, particularly antidepressant and/or mood-stabilizing agents. We will give particular attention to investigators with research interests in genome biology, studies of complex traits or human disease in model organisms, and translational research. Research space and appropriate startup funds are available. Applicants must have a demonstrated potential to develop an independently funded research program. Interested applicants should send copies of curriculum vitae, a short summary of research plans, the names and addresses of three references, and arrange for these letters to be sent separately to: **Emil F. Coccaro, Chairman, Department of Psychiatry, The University of Chicago, 5841 S. Maryland Avenue, Chicago, IL 60637 (e-mail: ecoccaro@yoda.bsd.uchicago.edu).** *The University of Chicago is an Affirmative Action/Equal Opportunity Employer.*

POSTDOCTORAL RESEARCH ASSOCIATE–RIPARIAN ECOLOGY. Incumbent will be stationed at Montana State University and participate with a research team conducting a long-term study on riparian ecology in Grand Teton National Park. The position will be renewed yearly for upwards of three years. Starting date, March 2005 to June 2005; salary $38,000 to $40,000 per year plus benefits. Contact: **Bruce Pugesek (telephone: 406-994-6144, or e-mail: bruce_pugesek@usgs.gov).** To apply send curriculum vitae, three letters of recommendation, and representative publications by February 15, 2005, to: **Bruce Pugesek, U.S. Geological Survey-Northern Rocky Mountain Science Center, 229 AJM Johnson Hall, P.O. Box 173492, Bozeman, MT 59717-3492.**

ASSISTANT/ASSOCIATE/FULL PROFESSOR (TWO POSITIONS)
Medicinal Chemistry
Department of Pharmaceutical Sciences

The Department of Pharmaceutical Sciences in the School of Pharmacy invites applicants for two tenure-track faculty positions available in fall 2005 at the Assistant, Associate, or Full Professor level in the area of drug discovery and design. Specific areas of interest include, but are not limited to, bioorganic synthetic chemistry/neurochemistry, computational tools to model and analyze biological systems, in vivo imaging, and structural biology. The successful applicant's research program will complement the interests of the faculty in the medicinal chemistry discipline in the Pharmaceutical Sciences Department (**website: http://www.pharmacy.uconn.edu**), which includes a focus on the use of structural biological approaches to identify and characterize novel drug target sites.

The candidate will be expected to establish and maintain an independent extramurally funded research program and participate in the Department's graduate and undergraduate teaching. Applicants must possess a Ph.D. degree or equivalent. Candidates for Associate or Full Professor are expected to have a currently funded active research program. Review of applications will begin January 20, 2005, and the search will continue until the positions are filled. Applications should include curriculum vitae, a brief statement of research and teaching interests, and names of three references. Application materials should be sent to: **Dr. David Grant, Chair, Medicinal Chemistry Search Committee, (Search#05A251 Assistant Professor), (Search#05A250 Associate/Full Professor), University of Connecticut, Department of Pharmaceutical Sciences, 372 Fairfield Road, Unit 2092, Storrs, CT 06269.**

BIOLOGY EDUCATOR

Tenure-track **ASSISTANT PROFESSOR.** Ph.D. in biology or subdiscipline in biology completed by July 31, 2005. Teaching experience and either broad training or experience in biology required. Preference given to applicants with demonstrated interest in pedagogy, K-14 education, a strong commitment to college teaching, prior teaching at the college level, and experience working with diverse groups. Duties may include teaching introductory biology; teaching courses in area of specialization; engaging in scholarly activities; advising students planning to teach K-14; serving on Department and University committees; engaging in community service; and academic advising; participation in Department and University programs designed to recruit and retain students in science and science education, and supervising undergraduate and Master's research. Submit curriculum vitae, all transcripts, names and telephone numbers of three references, and statements of teaching and scholarly interests. Applicants must also have three letters of recommendation sent to: **Nicholas Ewing, Chair, Biological Sciences, California State University, 6000 J Street, Sacramento, CA 95819-6077. Website: http://www.csus.edu/bios/.** Applications should be received by February 7, 2005, to ensure consideration. Position open until filled. *Affirmative Action/Equal Employment Opportunity.*

RESEARCH ASSOCIATE POSITION

Applications are invited for a Research Associate position in the Department of Chemistry and Chemical Biology at Stevens Institute of Technology, Hoboken, New Jersey 07030. Candidates must have a Ph.D. degree in cell biology, molecular biology, or a closely related field. Background in molecular biology techniques, Xenopus oocyte expression system, or confocal microscopy will be advantageous. Send resume and names of three references to: **Dr. Sunil Saxena** at **e-mail: ssaxena@stevens.edu** or **fax: 201-216-8240.**

COURSE

MBL
Marine Biological Laboratory
7 MBL Street • Woods Hole • MA • 02543

2005 Microscopy Courses

Analytical & Quantitative Light Microscopy
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This comprehensive course provides an in-depth examination of the theory of image formation and the application of video methods for exploring subtle interactions between light and the specimen.

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October 11 - October 20, 2005
APPLICATION DEADLINE: June 20, 2005

This course will enable the participant to obtain and interpret microscope images of high quality to perform quantitative optical measurements and to produce video and digital records for documentation and analysis.

For further information and applications, visit:
www.mbl.edu/education
or contact: Carol Hamel, Admissions Coordinator,
(508)289-7401 admissions@mbl.edu
Women and minorities encouraged to apply.
The MBL is an EEO/Affirmative Action Institution

FELLOWSHIPS

Monterey Bay Aquarium Research Institute

2005 POSTDOCTORAL FELLOWSHIPS

Founded in 1987 and supported by the David and Lucile Packard Foundation, The Monterey Bay Aquarium Research Institute (MBARI) is a non-profit oceanographic research institute, dedicated to the development of state-of-the-art instrumentation, systems, and methods for scientific research in the oceans. MBARI's research center includes science and engineering laboratories, as well as an operations facility to support our research vessels and oceanographic equipment, including remotely operated and autonomous underwater vehicles. Located in Moss Landing, California, the heart of the nation's largest marine sanctuary, MBARI places a balanced emphasis on science and engineering, with established programs in marine robotics, ocean physics, chemistry, geology, and biology, as well as information management and ocean instrumentation research and development.

MBARI invites applications each year for several postdoctoral fellowships in the fields of biological, chemical, and physical oceanography, marine geology, and ocean engineering. Fellowships may require occasional trips to sea. Awards are typically for two years.

Candidates must complete their Ph.D. degree prior to commencing the two-year appointment between October 2005 and March 2006.

Application deadline: February 11, 2005. The final selection of candidates for these fellowships takes place in March. Selections will be announced in early April 2005.

Note: It is helpful for applicants to communicate with potential research sponsors at MBARI (**www.mbari.org/about/researchers.html**) for guidance on project feasibility, relevance to ongoing MBARI research, and resource availability.

Application requirements:
- Curriculum vitae
- Potential research goals at MBARI
- Supplemental Information online form (**www.mbari.org/oed/jobs/forms/postdoc_form.htm**)
- At least three professional letters of recommendation
- Succinct statement of the applicant's doctoral research

Competitive compensation and benefits package.
MBARI considers all applicants for employment without regard to race, color, religion, sex, national origin, disability, or veteran status.

Address your application to:
MBARI Human Resources
Job code: Postdocs-2005
7700 Sandholdt Road, Moss Landing, CA 95039-9644

Submit by e-mail to jobs@mbari.org,
or by fax to (831) 775-1620.

M B A R I

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The *Science* Meetings & Announcements Database

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WORKSHOPS

Second International Bioprinting, Biopatterning and Bioassembly Workshop
March 14-15, 2005
Charleston, South Carolina, USA

Workshop Goals
Assemble World Leaders To Present Their Latest Results And Assess Future Trends In The New And Emerging Fields Of Bioprinting, Biopatterning, And Bioassembly; Explore New Applications And Promote International Collaborations And Technology Transfer Through Industrial Partnerships In R&D

Keynote Speakers
Teruo Okano (Japan) "Tissue Bioassembly Using Cell Sheet Technology"
Ben Hsieh (USA) "Bioprinting Using Disposable Piezoelectric Ejector"
Cerasela Dinu (Germany) "Towards Molecular Manufacturing"
Wei Sun (USA) "Computer-Aided Tissue Engineering"
Thomas Boland (USA) "Drop-on-demand Bioprinting"

Scientific Sessions
Molecular Bioprinting and Biopatterning; Cell Bioprinting and Biopatterning; 3D Tissue Bioassembly and Organ Printing; Printing Scaffolds for Tissue Engineering; Photo-patterning and Laser-based Bioprinting; Bioprinting and Bioassembly Enabling Technologies

Special Sessions
- Towards Industrial Bioprinting and Bioassemby: Role of Academic-Industrial Partnership
- How to Train Specialists in Bioprinting, Biopatterning and Bioassembly?
- Future of Bioprinting, Biopatterning and Bioassembly Technologies

The number of workshop participants is limited. Student participation is welcome and encouraged.

Contact person for interested participants, sponsors and media: **Vladimir Mironov M.D., Ph.D., Shared Tissue Engineering Laboratory, Department of Cell Biology and Anatomy, Medical University of South Carolina, Charleston, SC 29425, USA; Tel: 843-792-7630; Fax: 843-792-0664; e-mail: mironovv@musc.edu; website: http://www2.umist.ac.uk/material/bioprint/.**

POSITIONS OPEN

TENURE-TRACK POSITION AND RESEARCH DIRECTOR
School of Podiatric Medicine
Temple University

Temple University School of Podiatric Medicine (TUSPM) is seeking an established, independent investigator for a tenure-track **FACULTY POSITION** to provide scientific leadership in the areas of diabetes and wound care. Qualified applicants must have a Ph.D. or equivalent degree and an outstanding research record. The successful applicant will be expected to establish an independent research program as well as direct the overall research efforts at TUSPM. Candidates should provide curriculum vitae, statement of current research, and a brief description of their long-range goals to **e-mail: jburke@tuspm.temple.edu** or to:

James P. Burke, Ph.D.
Associate Dean for Academic Affairs
Temple University
School of Podiatric Medicine
8th Street at Race Street
Philadelphia, PA 19107

Applications will be reviewed upon receipt and accepted until the position is filled. *Temple University is an Affirmative Action/Equal Opportunity Employer. Women and Minorities are encouraged to apply.*

BIOCHEMIST
Whitman College

Biochemist, Whitman College: One-year sabbatical leave replacement position as **VISITING ASSISTANT PROFESSOR** beginning August 2005. Responsibilities include teaching in the biochemistry, biophysics, and molecular biology major program (biochemistry lecture and laboratory, senior seminar and supervision of student research) and core introductory courses in chemistry. Preference will be given to candidates who have completed their Ph.D. Send application letter, curriculum vitae, three original letters of recommendation, statement of teaching philosophy, and evidence of teaching excellence and official undergraduate and graduate transcripts to: **James E. Russo, Biochemistry Search, Whitman College, 345 Boyer Avenue, Walla Walla, WA 99362.** No electronic submissions accepted. Deadline: February 1, 2005. Additional information about Whitman College at **website: http://www.whitman.edu,** or contact: **James Russo** at **telephone: 509-527-5228; e-mail: russo@whitman.edu.** *Applicants who would enrich the diversity of the campus community are strongly encouraged to apply.*

MEDICAL WRITER

Physicians' Education Resource (PER) is seeking a medical writer/editor to join its team. PER is a medical education company, located in Dallas, Texas, specializing in the field of oncology. Successful candidates will be responsible for writing manuscripts from original data, reporting highlights from cancer meetings, creating slide sets for pharmaceutical companies, and editing and rewriting author-submitted manuscripts. This full-time position requires a Ph.D. in a biomedical science. Send resume and salary requirements to: **Barb Schmaedeke, Human Resources Director, 3535 Worth Street #185, Dallas, TX 75246. E-mail: hr@perlp.com.**

RESEARCH SCIENTIST. Ph.D. (all but dissertation) in dairy or food science or related field. Three years experience, including experience with: chemical microbiological and rhelogical analysis of dairy foods; conducting research projects using experimental design; using statistical analysis software to analyze and interpret research results; isolating, identifying, and characterizing proprietary strains of dairy cultures and their commercial use. Theoretical knowledge and/or practical experience in the composition, properties, formulation, and manufacture of milk and dairy products. Proficient in using Excel, Microsoft Access, and Oracle. Send resumes to: **Jamie Spangler, 800 Lincoln Street, S.W., Le Mars, IA 51031.** *Equal Opportunity Employer.*

FACULTY POSITION
University of Louisville
Center for Oral Health and Systemic Disease

The University of Louisville Center for Oral Health and Systemic Disease invites applications for senior tenure-track faculty appointments at the level of **ASSOCIATE** or **FULL PROFESSOR.** Candidates will have an established research program preferably in the areas of inflammation/immunology or host-pathogen interactions. However, candidates with research experience in any area related to oral health are encouraged to apply. Successful candidates will have a history of previous funding, a strong publication record, and will be expected to maintain an independent and innovative research program that attracts extramural funding. The Center for Oral Health and Systemic Disease is a multidisciplinary research group that is expanding its research strengths in the areas of oral and systemic inflammatory and immune responses, microbial pathogenesis, epidemiology, genetics, and pathology of both oral and systemic diseases. The Center encourages and offers extensive opportunities for collaboration with scientists in the University of Louisville Health Sciences Center, an active research environment supported by numerous research centers (e.g., microarray, proteomics, transgenics).

Applicants should send curriculum vitae, a description of research activities and plans, and the names of three references to: **Dr. Denis F. Kinane, Associate Dean for Research and Enterprise, Director, Center for Oral Health and Systemic Disease, University of Louisville School of Dentistry, 501 S. Preston Street, Louisville, KY 40292. E-mail: dfkina01@louisville.edu.**

The University of Louisville is an Equal Employment Opportunity/Affirmative Action Employer. Women and minorities are encouraged to apply.

The Department of Microbiology, Immunology, and Parasitology at Louisiana State University Health Sciences Center (LSUHSC) invites applications for two tenure-track positions at the **ASSOCIATE PROFESSOR** level. One is for an outstanding candidate with a strong interest in bacterial causes of infectious diseases. The other is for an immunologist with a strong interest in infectious disease and/or cancer. Successful candidates will be active members of the Stanley S. Scott Cancer Center. The Department includes 15 funded faculty members actively involved in host-pathogen research. Applicants should have a Ph.D. or equivalent degree with postdoctoral experience and an established, funded research program. Send curriculum vitae, statement of research interests, and three letters of reference to: **Dr. Ronald B. Luftig, Chair of the Search Committee, Microbiology, Immunology, and Parasitology, Louisiana State University Health Sciences Center, 1901 Perdido Street, Box P6-1, New Orleans, LA 70112-1393.** *LSUHSC is an Equal Employment Opportunity/Affirmative Action Employer.*

SYSTEMATIC BOTANIST (RESEARCH BOTANIST)
Smithsonian Institution
National Museum of Natural History

The Department of Botany seeks a Systematic Botanist for a full-time research position. Candidates should have demonstrated expertise in innovative as well as conventional systematics and a proven record of scientific achievement in their research specialty and expertise. The position is initially a four-year term appointment and will be filled at the GS-12 entry level (salary range of $60,638 to $78,826 commensurate with experience). Applicants must have demonstrated ability to establish an externally funded research program, and to conduct active botanical fieldwork and/or collection building. Further important details for this position can be found at **websites: http://www.sihr.si.edu** or **http://www.nmnh.si.edu/botany,** reference announcement number 04AD-1416. *The Smithsonian Institution is an Equal Opportunity Employer.*

The Department of Microbiology and Molecular Biology at Brigham Young University (BYU) announces the availability of a permanent (continuing faculty status track) **FACULTY POSITION.** Review of applications will begin February 1, 2005, and continue until the position is filled. Applicants should have a Ph.D. degree and postdoctoral experience. Candidates are expected to develop a strong teaching capability in molecular biology and have a research emphasis focusing on molecular mechanisms of gene expression. Specific areas of interest include, but are not limited to, microbial genetics, the study of transcriptional or posttranscriptional regulation with an emphasis on protein-protein or protein-nucleic acid interactions, or a proteomics-based approach to study regulatory networks within cells. Candidates must demonstrate a high potential for establishment of an externally funded research program. Apply online at **website: http://yjobs.byu.edu** through faculty application, attach curriculum vitae, and one-page statements of teaching philosophy and of research interests and goals. Contact: **Dr. Byron Murray, Chair Search Committee, Department of Microbiology and Molecular Biology, Brigham Young University, Provo, UT 84602. Telephone: 801-422-2889; e-mail: byron_murray@byu.edu.** Additional departmental information is available at **website: http://mmbio.byu.edu.** *BYU is an Equal Employment Opportunity Employer. Preference is given to qualified members in good standing of the sponsoring church, The Church of Jesus Christ of Latter-day Saints.*

POSTDOCTORAL FELLOW IN POPULATION BIOLOGY–The Center for Population Biology (CPB) at U. C. Davis (UCD) invites applications for a Postdoctoral Fellowship in population biology, broadly defined to include ecology, systematics, population genetics, and evolution. The position is for two years, subject to review after one year, and can begin as early as 1 July 2005. It has an annual salary of $35,000 plus benefits, and $4,000 per annum in research support. The Fellow will be a fully participating member in the Center for Population Biology, and will be expected to have an independent research program that bridges the interests of two or more CPB laboratory groups. For more information about UCD programs in population biology, see **website: http://www.cpb.ucdavis.edu.** Interested candidates should submit a cover letter, curriculum vitae, a short (one to two page) description of research accomplishments, and a short (one to two page) description of proposed research indicating potential faculty mentors, and copies of two publications at **website: http://www2.eve.ucdavis.edu/jobs/** all as PDFs. You should also have three letters of reference sent by e-mail to: **Karen Reid** at **e-mail: kareid@ucdavis.edu;** please follow instructions at the website. Application evaluation will begin on January 14, 2005. *The University of California is an Affirmative Action/Equal Opportunity Employer with a strong institutional commitment to the development of a climate that supports equality of opportunity and respect for differences.*

POSTDOCTORAL RESEARCH ASSOCIATE

The Department of Ecology and Evolutionary Biology is seeking a Postdoctoral Research Associate to assist in teaching a fall semester course in human morphology. The second semester and summer will be available for full-time research. This position is available for the 2005-2006 academic year with a possibility for a two-year renewal. Applicants must have experience with a cadaver-based gross anatomy course, a Doctorate, and potential for excellence in teaching and research in evolutionary morphology.

Applicants should submit a resume that includes teaching experience, research interests, list of publications, up to three representative reprints, and names of three referees, to be received by February 1, 2005, sent to: **Dr. S.M. Gatesy, Box G-B209, Department of Ecology and Evolutionary Biology, Brown University, Providence, RI 02912.**

Applications received by February 1, 2005, will receive full consideration.

Brown University is an Equal Opportunity/Affirmative Action Employer.

POSITIONS OPEN

City of Hope
Where the Power of Knowledge Saves Lives
www.cityofhope.org

POSTDOCTORAL FELLOW POSITION

A Postdoctoral position is available in the Department of Molecular Genetics: Nano Technology, **Steve Sommer, M.D., Ph.D., Chair.** The goal of this funded project is to efficiently scan for unknown mutations by electrophoresing DNA through artificial gels composed of silicone nanotubes: "electrophoresis on a chip." The first year will be spent at the nano fabrication facility at Cornell University; and the second year will be spent in enhancing and validating the device at City of Hope. Please send a Statement of Goals, curriculum vitae, and the contact information of three references to: **c/o Academic Personnel Office, Beckman Research Institute of the City of Hope National Medical Center, 1500 E. Duarte Road, Duarte, CA 91010. E-mail: iabich@coh.org.** *Equal Opportunity Employer.*

POSTDOCTORAL POSITIONS are available in the Center for Oral Biology at the University of Rochester Medical Center to study either: (1) antibiotic stress response in Streptococcus pneumoniae (*J. Bacteriol.*, in print, 2004, **Dr. Wolfgang Haas**); (2) molecular genetic basis of mammalian craniofacial development and birth defects (e.g., *Proc. Natl. Acad. Sci.* **101:**7022–7027, 2004; *Development* **131:**3207–3216, 2004, **Dr. Rulang Jiang**); and (3) regulation of Muc19 apomucin gene expression and elucidation of the sld mutation (*Physiol. Genomics,* **14:**95, 2003 and **19:**303, 2004, **Dr. David Culp**). Molecular biology experience required. Salary commensurate with experience. Send curriculum vitae, two letters of reference, and one page statement of career aspirations to: **Wendy Keck, Center for Oral Biology, Box 611, 601 Elmwood Avenue, Rochester, NY 14642. E-mail: wendy_keck@urmc.rochester.edu.** Center information is available at **website: http://www.urmc.rochester.edu/Aab/Oralbio/.** *The University of Rochester Medical Center is an Affirmative Action and Equal Opportunity Employer and Educator.*

ASSISTANT RESEARCH SCIENTIST

An Assistant Research Scientist position is available in the Laboratory of **Dr. Nicholas Zavazava,** Director of Transplantation Research at the University of Iowa (*Nat. Med.* **8:**171–178; *Nat. Med.* **2:**1005–1010; *Blood* **99:**3286–3292). Applicants must have a Ph.D. and or M.D. and should have experience in cell biology, molecular biology, or immunology. The laboratory currently focuses on embryonic stem cell research in animal models. Applications, including curriculum vitae and bibliography, summary of past accomplishments, and names of three references should be sent to: **Nicholas Zavazava, M.D., Ph.D., University of Iowa, Department of Internal Medicine, 200 Hawkins Drive, Iowa City, IA 52242. Telephone: 319-384-6577; e-mail: nicholas-zavazava@uiowa.edu.**

The University of Iowa is an Equal Opportunity and Affirmative Action Employer. Women and minorities are strongly encouraged to apply.

POSTDOCTORAL POSITION

A Postdoctoral position is available to study the molecular mechanism of axonal guidance in the spinal cord. Work in our laboratory is directed toward the identification and analysis of molecules that regulate formation of axonal pathways. Candidates with experience in neuroanatomy and tissue culture are encouraged to send their curriculum vitae and the names of three references to: **Dr. Renping Zhou, Laboratory for Cancer Research, Rutgers University, School of Pharmacy, 164 Frelinghuysen Road, Piscataway, NJ 08854-8020. Fax: 732-445-0687; e-mail: rzhou@rci.rutgers.edu.**

POSITIONS OPEN

POSTDOCTORAL POSITION SYSTEMS NEUROSCIENCE University of Tennessee Health Science Center

A Postdoctoral position is available in the Department of Anatomy and Neurobiology of the University of Tennessee Health Science Center in Memphis, Tennessee, to study the dynamics of neuronal interaction during voluntary movement. Applicant should have a background in systems-level neuroscience and in vivo electrophysiology.

Please send your curriculum vitae and the names and e-mail addresses of two references to: **Detlef Heck, Ph.D., University of Tennessee Health Science Center, Department of Anatomy and Neurobiology, 855 Monroe Avenue, Memphis, TN 38163, U.S.A. E-mail: dheck@utmem.edu.**

The University of Tennessee is an Equal Employment Opportunity/Affirmative Action/Title VI/Title IX, Section 504/ADA/ADEA Employer.

FELLOWSHIPS

ROBERT M. WALKER POSTDOCTORAL FELLOWSHIP in experimental space sciences. In tribute to its founding Director, the McDonnell Center for the Space Sciences at Washington University in St. Louis invites exceptionally well-qualified individuals in any area of experimental space sciences to apply for this prestigious postdoctoral position.

The McDonnell Center involves faculty chiefly from the Departments of Physics and Earth and Planetary Sciences, and its faculty members are involved in a broad range of theoretical and experimental space science activities (see **website: http://mcss.wustl.edu/**). Investigations involve both space-borne and balloon-borne experiments, as well as experiments carried out with state-of-the art laboratory instrumentation (e.g., the NanoSIMS, **website: http://presolar.wustl.edu/nanosims/**). The Walker postdoctoral Fellow would be based in one of these two Departments, and interact with members of the McDonnell Center faculty from both departments. The appointment would nominally be for two years, with a third year possible. The annual salary is $65,000, with an annual research and travel budget of $15,000.

A Ph.D. or equivalent in a field related to the space sciences is required. Please submit a cover letter, brief statement of research interests, curriculum vitae (including a list of publications). Please also request that three letters of reference be sent to us on your behalf.

Applications and reference letters should be sent to: **Dr. Roger J. Phillips, Director, McDonnell Center for the Space Sciences, Washington University, Campus Box 1169, One Brookings Drive, Saint Louis, MO** 63130-4899. Inquiries can be directed by **e-mail: phillips@wustite.wustl.edu.**

Employment eligibility verification required upon employment. Applications will be considered until the position is filled, but priority will be given to those received by March 31, 2005. *Women and minorities are encouraged to apply. Washington University is an Equal Opportunity/Affirmative Action Employer.*

PEDIATRIC NEUROMUSCULAR FELLOWSHIP with a special emphasis on spinal muscular association offered by Columbia University and the Neurological Institute of New York. The program emphasizes (1) comprehensive clinical training, (2) interpretation of muscle and nerve biopsies, (3) electrode monitoring procedure, and (4) research training. Fellows spend 75 percent of their time on clinical activities and 25 percent on research activities during the first year. In the second year, research time is 75 percent. Applicants must be graduates of an Accreditation Council for Graduate Medical Education-approved Child Neurology residency program and have a U.S. medical license. Post-graduate year level salary. Contact: **Dr. Darryl C. De Vivo, 710 W. 168th Street, New York, NY 10032. E-mail: dcd1@columbia.edu. Telephone: 212-305-2544.** *Columbia University is an Equal Employment Opportunity Employer.*

POSITIONS OPEN

POSTDOCTORAL AND CLINICAL FELLOWSHIPS at the National Institutes of Health U.S. Department of Health and Human Services

Website: http://www.training.nih.gov

NIH is dedicated to building a diverse community in its training and employment programs.

POSTDOCTORAL POSITIONS Immunology/Virology/ Vaccine Development

NIH-funded positions in the laboratory of **Dr. Norman Letvin** at Harvard Medical School to explore the immune control of HIV replication and novel HIV vaccine strategies in nonhuman primate models. Projects involve basic and translational science. Candidates must have a Ph.D. and/or M.D./D.V.M. and have prior research experience in immunology or virology. Submit letter of interest, curriculum vitae, and names of three references by **e-mail: nsicilia@bidmc.harvard.edu** or by **fax: 617-667-8210.**

TRAINING

POSTDOCTORAL FELLOW Training Program in Cardiovascular Signaling University of Miami School of Medicine

We are seeking outstanding postdoctoral candidates for our 29-year-old NIH-funded Training Program in Cardiovascular Signaling. The Program's more than 40 students and postdoctoral fellows contribute to the creative and stimulating scientific environment at the University of Miami School of Medicine. We have a well-funded faculty with diverse research interests including: (1) regulation of cardiac growth, differentiation and apoptosis; cellular and genetic mechanisms of heart failure and cardiomyopathies. (2) regulation of cardiac and skeletal muscle contraction. (3) intracellular trafficking and nuclear pore function; cell cycle control in cancer; morphogenesis and cytoskeletal dynamics. (4) mechanisms of steroid receptor and G protein-mediated signal transduction; structure/function analysis of membrane receptors. (5) molecular biology of neuronal differentiation, axon growth, and synapse formation. (6) genomics, bioinformatics, and genetic models of signal transduction. (7) discovery and target identification of natural therapeutics; stem cell therapy for cardiac disease.

All applicants who are either U.S. citizens or permanent residents should send electronic copies of their curriculum vitae, a brief description of their research background, and three reference letters to **e-mail: spizano@med.miami.edu.** *The University of Miami School of Medicine is an Equal Opportunity/Affirmative Action Employer.*

MARKETPLACE

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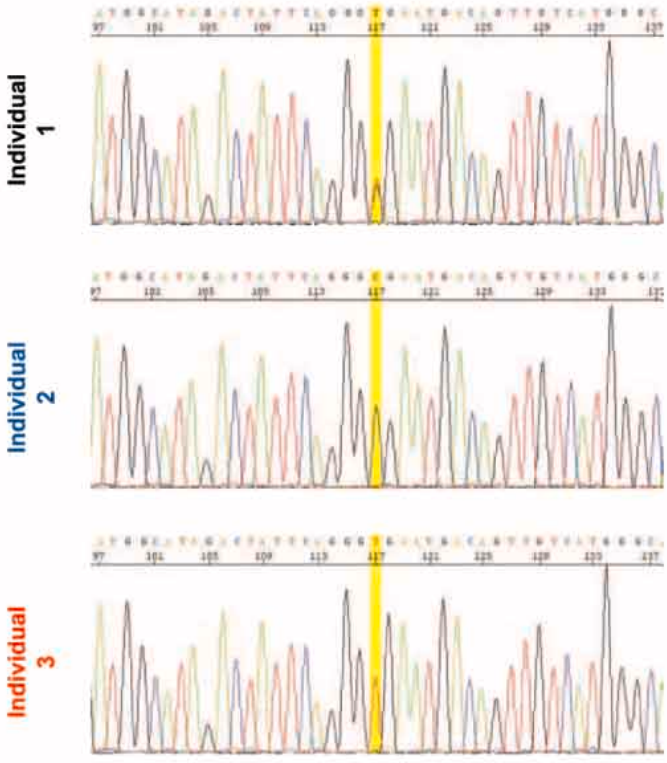
SNP discovery for 1¢ per base analyzed



A 3.2 Kb region of human chromosome 6 was sequenced for 32 individuals. The above figures show DNA sequencing electropherograms in the region of a polymorphism (highlighted in yellow) for the first 3 individuals. Individual "1" is Heterozygous C/T at this site, individual "2" is Homozygous C, and individual "3" is Homozygous T. Below are shown aligned DNA text data in this same region for the first 7 individuals.

```
Ref. Seq.  : ...ATGGCATAGACTATTCAGGGCGAATGACAGTTGTCATGGGC...

Individual 1: ...ATGGCATAGACTATTCAGGGYGAATGACAGTTGTCATGGGC...
Individual 2: ...ATGGCATAGACTATTCAGGGCGAATGACAGTTGTCATGGGC...
Individual 3: ...ATGGCATAGACTATTCAGGGTGAATGACAGTTGTCATGGGC...
Individual 4: ...ATGGCATAGACTATTCAGGGTGAATGACAGTTGTCATGGGC...
Individual 5: ...ATGGCATAGACTATTCAGGGCGAATGACAGTTGTCATGGGC...
Individual 6: ...ATGGCATAGACTATTCAGGGYGAATGACAGTTGTCATGGGC...
Individual 7: ...ATGGCATAGACTATTCAGGGCGAATGACAGTTGTCATGGGC...
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